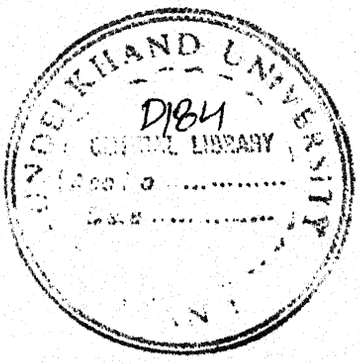
IMMUNOGLOBULIN-M ESTIMATION AND C-REACTIVE PROTEIN DETECTION IN NEONATAL SEPTICEMIA

THESIS

FOR

DOCTOR OF MEDICINE

(PEDIATRICS)



BUNDELKHAND UNIVERSITY
JHANSI (U.P.)

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CERTIFICATE

This is to certify that the work entitled " ***Immunoglobulin-M estimation and C-reactive protein detection in neonatal septicemia*** " has been carried out by ***Dr. Rachna Kankani*** in the Department of Pediatrics, M.L.B. Medical College, Jhansi.

She has put in the necessary stay in the Department as per University regulations.

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CERTIFICATE

This is to certify that the work entitled ***"Immunoglobulin-M estimation and C-reactive protein detection in neonatal septicemia "*** which is being submitted as a thesis for M.D. (Pediatrics) Examination 2005 of Bundelkhand University, Jhansi, has been carried out by ***Dr. Rachna Kankani*** under my direct supervision and guidance. The techniques embodied in the thesis were undertaken by the candidate herself and the observations recorded have been checked and verified by me from time to time.

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Dedicated

To My Parents and My Husband

with a deep sense of respect

and love

Acknowledgement

On presenting the thesis work, no words, no calligraphic, no adjectives can completely express the profound sense of gratitude and thanks giving for those who have been instrumental in giving their assiduous efforts to its present form.

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Rachna

Date : 29 / 10 / 04

Dr. Rachna Kankani

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Introduction

Introduction

The term sepsis neonatorum is applied when there are various systemic signs of sepsis along with bacteremia in the first four weeks of life (Mathur et al, 1996). This is a generic term which incorporates neonatal septicemia, pneumonia, meningitis and urinary tract infections which may manifest clinically in isolation or in different combinations. In neonatal septicemia, the newborn has overwhelming bacteremia with poor localization of infection into any particular organ system. Newborns are prone to develop serious infections from the organisms that are non pathogenic for older people and also signs of infections both local and systemic may be absent, minimal or hard to detect.

Bacteremia signifies the presence and circulation of bacteria in the blood. Bacteremia may be:

- Transient : with no signs of infection and occurring in healthy neonates.
- Occult : with symptoms of infection but no focus.
- Intermittent : occurring with intermittent discharge from a focus of infection.

- Persistent : recognized focus of infection with continuous invasion of microorganisms in the blood stream (Campas et al, 1994).

Septicemia may be brief until the organisms are clinical or a more constant and recurrent event (Phillips and Eykyn 1990).

Incidence of neonatal septicemia has changed a little in the past 50 years in our country, affecting one to five infants per 100 live births, however, the mortality rate for early onset septicemia has declined considerably.

The mortality rate in the very low-birth-weight infants has not exhibited a similar decline and is still ten-fold greater than that of neonates with higher birth weight. Septicemia remains a significant cause of morbidity and mortality in the newborns; more so in the developing countries due to delivery and post natal follow up in an unclean environment, leading to more chances of contamination with infective organisms (Khatua et al, 1986). In developing countries, sepsis is the commonest cause of mortality responsible for 30 – 50% of five million total neonatal deaths each year. It is estimated that almost 20% of all neonates develop infection and approximately 1% die of serious infections. Morbidity and mortality rate from neonatal sepsis is still high in spite of development of broad spectrum antibiotics and advances in life support therapy (Stoll et al, 1997).

Neonatal septicemia, its high incidence and grave prognosis, inspite of the modern antibiotics has been a challenge for all times (Mishra et al, 1985). The pattern of organisms responsible for neonatal septicemia have been constantly changing. The frequent emergence of resistant bacteria makes the problem more difficult. (Khatua et al, 1986).

Systemic bacterial disease occurs in 1 to 10 cases per thousand live births, depending on such factors as the rate of prematurity, predisposing maternal conditions and the extent of life support procedures required postnatally (Siegel and McCraken, 1981).

Neonatal septicemia occurs in 10.97 to 15.5 per thousand live births in India (Khatua et al, 1986, Mondal et al, 1991) and is the leading cause of neonatal mortality; next only to perinatal hypoxia (Thora et al, 1986). It is responsible for one quarter to nearly half of all neonatal deaths, even with the most intensive management (Singh et al, 1988).

The clinical diagnosis of neonatal septicemia in many situations is difficult, as it presents with non-specific symptoms and signs (Siegel and McCraken 1981). One of the most difficult task is to clinically differentiate between septicemic and non-septicemic cases. This is because several conditions like birth asphyxia, hypoglycemia, hypothermia, pre-maturity, and intracranial hemorrhages have clinical features similar to septicemia, or many times septicemia may be

associated with any of the above mentioned conditions (Chandna et al, 1988).

As the clinical diagnosis of septicemia is very difficult task and symptoms are non-specific and subtle, one has to depend on laboratory investigations for a firm diagnosis. The definitive diagnosis of septicemia is made by a positive blood culture which requires a minimum period of 48 to 72 hours and yields a positive result in 30 – 70% cases (Chandna et al, 1988). Repeated samples are necessary to confirm an isolate as the causative agent specially so when organism like staphylococcus epidermidis and non-fermenting gram negative bacilli are involved. This may not always be possible in neonates. Facilities for culture of fastidious organisms and anaerobes, which form a significant part of the causative agents, may not be available in all laboratories, more than 48 – 72 hours may be needed for the isolation of such organisms. Thus, the above mentioned factors make it imperative to consider non-culture diagnostic methods in neonatal septicemia. Thus, the need for a rapid diagnosis of neonatal septicemia cannot be over emphasized. A number of independent observers have suggested that several different laboratory determinations are individually helpful in detecting bacterial infection in the newborn infant. The rapid tests help to diagnose bacteremia faster, so that suitable antibiotic treatment can be started at the

earliest to prevent morbidity and mortality and also to prevent unnecessary antibiotic administration to neonates in the absence of bacteremia (Parikh and Singh 1995).

Among these immunoglobulins – M estimation and C-reactive protein (CRP) test has been employed as adjuncts to blood culture in neonatal septicemia.

C-reactive protein, an acute phase reactant is found to be elevated in neonatal infections and its value return to normal with clinical improvement and hence has been designated as simple effective and early test for the diagnosis of neonatal sepsis.

Immunoglobulins constitute 20 – 25% of the total serum proteins. Immunoglobulins are glycoproteins based on physico-chemical and antigenic differences five classes of immunoglobulins have been recognized IgG, IgA, IgM, IgD, IgE.

IgM is the oldest immunoglobulin class. It is also the earliest immunoglobulin to be synthesized by the fetus, beginning by about 20^{th} week of age. As it is not transported across the placenta, the presence of IgM in the fetus or newborns indicates intrauterine infection.

IgM is the first class of immunoglobulin synthesized in response to particulate antigens. IgM is multivalent and deals most efficiently with polyvalent antigens such as bacteria.

Aims

&

Objectives

Aims and objectives

1. To study the clinical profile of neonatal septicemia.
2. To study the diagnostic utility of immunoglobulin – M estimation and C-reactive protein detection in neonatal septicemia.
3. To evaluate the predictive value of IgM and C-reactive protein in outcome of neonatal septicemia.

Review Of Literature

Review of Literature

Sepsis is a Greek word meaning 'Putrefaction'. Septicemia is an ancient malady. Symptoms of septicemia have been described in the ancient Ayurvedic literature. The earliest documented case of septicemia occurred in a Chinese man, who died more than 2,100 years ago. An autopsy performed in 1975 on his well preserved body revealed that septic shock was the cause of his death (Shenep 1990).

P.A. Piorry in 1837 introduced the term "septicoemie", to describe the clinical condition of "putrid intoxication of the blood". At that time the etiology of this condition was unknown (Bulloch 1938). Shortly thereafter Schwann demonstrated that blood obtained directly from vessels would not putrefy if exposed exclusively to heated air. In 1860, Vander Broek, further advanced techniques for obtaining uncontaminated blood, by using a heated copper tube for collection (Shenep 1992).

In America, Oliver Wendell Holmes and in Veinna, Ignaz Philip Samelweiss in 1847, independently made the observations of high mortality among women hospitalized with puerperal fever. Both observed the death of a fellow physician who had been an infected subject. This experience brought attention to the fact that infection was being transmitted directly and both urged washing of hands and

changing of instruments while doing any procedure. These policies caused reduction in the maternal mortality from 11.4% in 1846 to 1.3% in 1848. Subsequently, Pasteur's discovery of bacteria prompted Lister to publish his antisepsis theory in mid of the nineteenth century. Later progress by German physician Kocher enabled to report 2.3% infection in clean wound.

Coze and Feltz reported the presence of bacteria in blood of patients suffering from typhoid fever, variola, scarlet fever, measles and puerperal fever in 1872 (Bulloch 1938). One of the earliest description of septicemia and its complications was on account of pseudomonas aeruginosa. Septicemia in an adult published by Brill and Silverman WA and Homan WE (1949) studied neonatal septicemia in New York.

Bacteremia

The term bacteremia was suggested by Ritche (1990); it denotes that bacteria are present in the blood but gives no indication as to whether they are harmless or likely to cause injury.

Septicemia

It implies the presence of organism and their toxin in the blood. The term is used when a patient shows severe toxemia, often with a

high swinging temperature, severe prostration and all constituent signs of infection according to Richie (1990).

CLASSIFICATION

Neonatal septicemia has been classified into different groups to facilitate a better understanding of the disease process and also to assist in a more efficient management of the newborn patient.

Neonatal septicemia has been variously classified based on different criteria's, such as the cause of septicemia, age at the onset and the mode of diagnosis.

According to the cause neonatal septicemia has been classified into:

1. Primary septicemia: Occurring in newborns with no evidence of local sepsis (Gluck et al, 1966).
2. Secondary septicemia: Associated with major anomalies; focal infections, debilitating illness or following surgery, or other manipulative procedures (Gotoff and Behrman 1970).

Depending on the age of neonate at the onset of septicemia, it has been divided into:

a. Early onset neonatal septicemia: Presents as a fulminant multisystemic illness, during the first week of life. These infants have a history of one or more significant obstetric complications

and many of the infants are premature or of low-birth-weight (Klein and Marcy 1995). In the West these infections are mostly caused by group B streptococci, while in other nurseries, most cases are due to gram negative organisms. Infection occurs either due to ascending infection following rupture of membranes or during the passage of baby through infected birth canal or at the time of resuscitation in the labour room (Singh 1991).

b. Late onset neonatal septicemia: Occurs after the first week of life. These infants may have a history of obstetric complications. Bacteria responsible for late onset sepsis include those acquired from the maternal genital tract and organisms acquired after birth from human contact or from contaminated equipment and materials (Klein and Marcy, 1995). Two-third cases of late onset septicemia are caused by gram negative bacteria (Singh 1991).

Mode of diagnosis forms the basis of yet another classification

Neonatal septicemia has been grouped into presumptive, probable and proven sepsis based on clinical manifestations, blood culture and laboratory evidence, other than blood culture. This classification is a reminder of the degrees of uncertainty of the diagnosis of neonatal septicemia (Moffet 1981).

Classification of neonatal sepsis based on the mode of diagnosis

Category	Clinical manifestation	Lab evidence other than blood culture	Blood culture
Presumptive sepsis	+	-	-
Probable sepsis	+	+	-
Proven sepsis	+	+	+

The incidence of neonatal septicemia has remained fairly constant over the last 5 – 6 decades.

ETIOLOGICAL AGENTS IN INDIA

Mishra et al (1985) studied 120 cases of blood culture positive neonatal septicemia at Bokaro. The common bacteria isolated were Escherichia coli, pseudomonas and staphylococcus. Incidence of gram negative infection was higher in babies under 2000 grams birth weight.

The various organisms responsible for neonatal septicemia and their changing sensitivity pattern were studied by Monga et al (1986) in Bombay among 392 cases. Gram negative organisms were isolated in the majority of blood cultures. Klebsiella, though showing a fall in incidence, still remained the commonest gram negative organism isolated. An increasing resistance of the isolates to penicillins and aminoglycoside was noted.

A study of 92 consecutive cases of neonatal septicemia by Khatua et al (1986) in Calcutta showed that blood culture was positive in 59.8% of cases. Gram negative organisms like Klebsiella, E.coli, Citrobacter, pseudomonas accounted for 76.2% of isolates and 23.76% were gram positive organisms, predominantly staphylococci and streptococci. Only one anaerobic peptococcus was isolated. All isolates were resistant to ampicillin and sensitive to gentamicin. Most organisms, except pseudomonas, were sensitive to cloxacillin.

Klebsiella was the most common organism (68.8%), in the analysis of 45 cases of culture positive neonatal septicemia, by Chugh et al (1988) in New Delhi. This remained the predominant organism in both early and late onset septicemia and was highly sensitive to gentamicin, streptomycin and cephaloridine.

Mondal et al (1991), in a study at Pondicherry of 100 neonates with sepsis (both hospital born and out born) identified a low birth weight and pre-maturity as important predisposing factor in both groups. Coagulase negative staphylococci, Klebsiella and Acinetobacter were the common causative organisms. All isolates were sensitive to gentamicin, whereas 75% of them were resistant to ampicillin.

ETIOLOGICAL AGENTS IN OTHER COUNTRIES

Work in various developing countries has shown a bacteriological profile similar to that in India.

Although gram negative bacteria were the predominant causative organisms during the 1960s in the West, recently the gram positive cocci, predominantly the group B streptococci have gained importance as the principal cause of neonatal septicemia, as reported by various authors.

In a review of 117 cases of septicemia at New Haven, U.S.A, Gluck et al (1966), observed a decline in the overall incidence of nursery – incurred primary septicemia. E.coli was the principal pathogen in patients with non-surgical problems (Primary septicemia). while in infants having surgical procedures the Klebsiella – aerobacter group accounted for the predominant organisms. Enterococcus was the only major gram positive pathogen.

Ohlsson et al (1986), studied 53 cases of neonatal septicemia in Riyadh, Saudi Arabia, where the common isolates were found to be E.coli, Klebsiella and S.aureus. Group B streptococci were isolated more often and Salmonella species less frequently than before.

Septicemia was the most frequent neonatal infection encountered by Chaudhary et al (1987) in Kabul. The organisms isolated were E.coli, Klebsiella and pseudomonas. One-third of the

neonates expired. Mortality was significantly higher among the prematures and neonates who were hospitalized within 24 hours of their birth.

In Jamaica, the most frequent cause of neonatal septicemia was group B streptococci, accounting for 35% of blood culture isolates in a study by Macfarlane (1987), followed by Klebsiella pneumoniae, S.aureus and others. Comparison of early and late onset disease, indicated an increased incidence of prematurity, prolonged rupture of membranes and respiratory distress in the former and a predominance of meningitis associated cases in the latter.

Vesikar et al (1988), reviewed history of proven cases of neonatal septicemia from seven Finnish hospitals. Mortality was higher in early onset septicemia and the leading cause was group B streptococci. The author concluded that early onset septicemia is probably intrauterine origin and caused by group B streptococci in one half of the cases, constituting the major form of neonatal septicemia in Finland.

A cohort study was conducted by Schuchat et al (1990) in Atlanta of infants with group B streptococcal disease, to determine the risk factors of infection. Black race, low birth weight and infants of teenage mothers had increased incidence of early onset disease.

Black infants had 35 times the risk of late onset disease than non-black infants had.

In a retrospective study of 231 cases of neonatal septicemia and meningitis by Tessin et al (1990) in Sweden the most common causative organisms were group B streptococci, S.aureus and aerobic gram negative rods, together isolated from 82% of patients. Group B streptococci were over represented in early onset infections in all birth weight infants and aerobic gram negative rods were the most common isolates from low birth weight infants with late onset infections. correlation of septicemia with level of serum immunoglobulin-M.

Studies were conducted by various authors to determine immunoglobulin-M (IgM) in neonates with acute infections.

Serum IgM and C-reactive protein were studied in 613 neonates and infants by Khan et al (1969). A high degree of correlation (75 – 85%) between high IgM levels (> 30 mg/dl) and infection was found. However, CRP was not found to be of value as a parameter of infection. Sudden death cases yielded higher IgM levels among both typical and atypical cases.

Prospective observations were made on 567 neonates by Korones et al (1969), to evaluate the association between elevation of IgM levels and occurrence of acute infections. Five clinical syndromes

of acute infection were studied. Abnormal elevations occurred in most infected infants, several days after the appearance of clinical signs.

Haider (1972), studied the dynamics of serum IgM in 36 neonates with different types of infection and 19 infants with probable infection. Serum IgM rose within two days of appearance of symptoms and the raise persisted as long as the infection was 'active', with the eradication of infection, the IgM levels tended to fall. This characteristic dynamic pattern of serum IgM was thought to be of considerable help in the diagnosis of infections in the neonates, especially the clinically inapparent and atypical varieties that may have serious sequelae later in life.

Determinations of C-reactive protein (CRP) and other acute phase reactants and hematological parameters have been evaluated by many authors, as an aid to the diagnosis of neonatal septicemia.

To better define the need for antibiotic therapy in neonatal sepsis, Philip and Hewitt (1980), evaluated several tests and found band to total neutrophil ratio, leukocyte count, latex C-reactive protein, ESR and latex haptoglobin to be most useful. When these five tests were applied early, 98% cases subsequently proven to have infection had two or more abnormal tests.

The neutrophil count, immature : total neutrophil ratio, C-reactive protein assays nitroblue tetrazolium test and an acridine orange

leukocyte cytospin test were evaluated for the diagnosis of neonatal bacteremia by Kite et al (1988). Combined results of the acridine orange leukocyte cytospin, C-reactive protein and nitroblue tetrazolium tests gave the best positive predictive value.

Blood culture was positive in 48% cases in the study by Chandna et al (1988), in which gram negative bacilli predominated. C-reactive protein test, total leukocyte count, ratio or band cells to total polymorphonuclear cells, buffy coat smear examination and gastric aspirate cytology were performed in all the cases. CRP was found to be the single most useful test with a high degree of sensitivity specificity and positive predictive accuracy.

Parikh and Singh (1995) evaluated 254 cases of clinically suspected bacteremia in neonates by blood culture and rapic diagnostic test viz. total neutrophil count, immature to total neutrophi ratio, C-reactive protein assay, nitroblue tetrazolium test and acridine orange stained buffy coat smear. E.coli was the commonest organism isolated. CRP had the highest sensitivity. The sensitivity, specificity and positive predictive accuracy of the combination of these tests (CRP, nitroblue tetrazolium test and acridine orange stained buffy coat smear) were better compared to the results of each test.

The pattern of bacteria responsible for neonatal sepsis is changing as reflected in the series of reports from various studies. In

the pre-antibiotic era, the commonest organisms causing septicemia were gram positive cocci, like streptococci (predominantly streptococcus pyogenes), S.aureus and pneumococci. With the introduction of antimicrobial agents, gram negative enteric bacilli, particularly E.coli, became the predominant cause of serious infection in the newborns with a corresponding decrease in septicemia caused by gram positive cocci. Most recent reports indicate that group B streptococci, E.coli, and S.epidermidis are important in neonatal sepsis in the United States.

EPIDEMIOLOGY

Incidence and mortality

The incidence of neonatal sepsis varies from less than 1 to 8.1 cases per thousand live births (Klein and Marcy 1995). The mortality ranges from 20% to more than 75% (Siegel and McCracken 1981). There is marked difference in both the incidence and mortality rates in the developed and developing countries.

The annual incidence of neonatal sepsis in Finland was reported to be 3 / 1000 live births, with an overall mortality rate of 23% (Veskari et al, 1985). In a study at Atlanta, U.S.A, during 1982 – 83 the incidence of early onset group B streptococcal disease was 1.09 / 1000 live births and late onset group B streptococcal disease was 0,57 cases / 1000 live births. The mortality for early onset disease was

11.27% (Schuchat et al, 1990). In Sweden the incidence was estimated to be 2.8 / 1000 live births, during a 12 years retrospective study, with a 15% mortality rate (Tessin et al, 1990).

The incidence of neonatal septicemia in Riyadh, Saudi Arabia, a developing country, was 2.5 / 1000 live births and the case fatality rate was 33% (Ohlsson et al, 1986). The incidence of group B streptococcal septicemia in Jamaica was 1.4 / 1000 live births, with an overall mortality rate of 36%. The mortality rate in early onset disease was 50%, whereas in late onset group B streptococcal disease it was 29% (Macfarlane 1987).

There is a wide range of variation in both the incidence and mortality rates of neonatal septicemia in different parts of our country. Guha et al in 1978, reported an incidence of neonatal septicemia in 27 / 1000 live births and a 24.3% case fatality rate. A 61.7% overall mortality with 71% mortality among infections with gram negative bacilli and 49% among infection with gram positive cocci was reported, from Bokaro in home delivered neonates (Mishra et al, 1985). In Calcutta, the incidence of neonatal septicemia was found to 6.55 / 1000 live births and the mortality rate was 57.6% (Khatua et al, 1986). In Bombay, the incidence of septicemia in the newborn was 26.3% of the total admissions to the neonatal intensive care unit (Monga et al, 1986). An incidence of 18.8 / 1000 live births with a

mortality rate of 23.9 / 1000 live births among the neonates with septicemia, was recorded by Singh and coworkers. In 1988, in New Delhi, Chugh et al reported a mortality of 53.3% from neonatal septicemia in New Delhi. In Pondicherry, the incidence of neonatal septicemia was 15.5 / 1000 live births, having a mortality rate of 32% among the out born babies and 10% among the hospital born babies (Mondal et al, 1991).

PREDISPOSING FACTORS

Host susceptibility, socioeconomic factors, obstetric and nursery practices and health and nutrition of the mothers are all important in the pathogenesis of the neonatal sepsis. Significant risk factors associated with pregnancy and delivery includes:

1. Low birth weight (< 2000 gms) or preterm.
2. Maternal peripartum infection.
3. Foul smelling and / or meconium stained liquor amnii.
4. Prolonged and / or premature rupture of membrane > 24 hrs.
5. More than three vaginal examinations during labour.
6. Prolonged and difficult delivery and instrumentation.
7. Birth asphyxia and difficult resuscitation.
8. Pathological evidences of funitis / presence of polymorphs in gastric aspirate.

Birth weight

Low birth infants have a high incidence of septicemia and mortality. It is important in both early onset and late onset sepsis (Schuchat et al, 1990).

Race

Black infants had a significantly higher incidence of septicemia than white infants. This may be due to higher rate of risk factors in the blacks (Klein and Marcy 1995).

Sex

A predominance of male infants is apparent in all studies of sepsis in the newborn infant. The usual male predominance in neonatal sepsis has suggested the possibility of a sex linked factor in host susceptibility. A gene located on the X-chromosome and involved with the function of the thymus or with synthesis of immunoglobulins has been postulated. The female has double the number of genes, affecting these factors and thus might possess a greater resistance to infection (Washburn et al, 1965).

Geographic factors

The bacterial etiology of neonatal sepsis varies from hospital to hospital and from one community to another. The bacteriology of neonatal sepsis in Western Europe and United States (predominantly group B streptococci) is different from that of tropical areas where gram negative bacilli remain the major pathogens (Klein and Marcy, 1995).

Socioeconomic factors

The life style patterns of the mothers, including cultural habits, housing, nutrition and level of income, appears to be an important factor in determining infants at risk of infection (Klein and Marcy, 1995).

Nursery outbreaks

The nursery is a small community of highly susceptible patients cared for by many adults, including mothers, nurses and physicians. Spread of micro-organisms to the infant occur by droplets from the respiratory tracts of parents, nursery personnel and other infants. Organisms may be transferred from infant to infant by the hands of the nursery personnel. The greatest hazard is however, an individual with an open or draining lesion.

Epidemics associated with contaminated equipments and solution, caused by proteus species, Klebsiella species, serratia marcescens, pseudomonas species and Flavobacteria, in addition to staphylococcal and group B staphylococcal epidermidis have been reported.

Antimicrobial agents, play a major role in the ecology of the microbial flora in the nursery. Extensive use of these drugs helps to eliminate the sensitive strains and allows proliferation of resistant stains. Thus, there is a selective pressure towards colonization by micro-organisms that are resistant not only to the antimicrobial agents used in the hospital, but because of cross resistance patterns, also to other similar drugs (Klein and Marcy 1995).

New strains of bacteria may appear in a nursery without changes in techniques, nursery practices or antimicrobial use. Marked annual variation in the prevalence of various bacteria colonizing the newborn infant have been noted (Klein and Marcy 1995).

Twin birth

The first born of the twins is at a higher risk of contracting ascending intrauterine infection, than the second born (Benirschke et al, 1960), and low birth weight twins are at a higher risk of infection than low birth weight singletons (pass et al, 1980).

Infections in the household

The number of contacts increase significantly when the newborn infant arrives home, allowing more opportunities for infection and disease in the neonate. Infection in infant have been associated with bites or licks of household pets (Klein and Marcy 1995).

PATHOGENESIS

The developing fetus is relatively protected from the microbial flora of the mother. Procedures disturbing the integrity of the uterine contents, such as aminocentesis, can however, permit entry of skin or vaginal organisms, causing amnionitis and secondary fetal infections (Klein and Marcy, 1995).

In most cases, the infant is colonized with the microflora of the birth canal during delivery. If delivery is delayed, however, vaginal bacteria may ascend and in some cases produce inflammations of the fetal membranes, umbilical cord or placenta (Benirschke et al 1960). Fetal infection can then result from aspiration of infected amniotic fluid, leading to still birth, premature delivery or neonatal sepsis (St. Geme et al, 1984).

Amniotic fluid is inhibitory to E.coli and other bacteria (Larsen et al, 1974), owing to the presence of lysozyme, transferrin or immunoglobulins (IgA and IgG) (Yoder et al, 1983).

The introduction of a small number of organisms would be unlikely to lead to significant infection, unless meconium or some other factors that promote bacterial growth were present (Klein and Marcy 1995).

Infection of the mother at the time of delivery, particularly genital infection can play a significant role in the development of infection in the neonate. Transplacental hematogenous infection during or shortly before delivery is possible, although it seems more likely that the infant is infected during passage through the birth canal (Klein and Marcy 1995).

Microorganisms acquired by the infant during birth colonize the skin and mucosa. Normal skin flora of the newborn infant includes, S.epidermidis, diphtheroides, and E.coli (Sacks et al, 1993). In most cases, bacteria proliferate at the initial site without causing illness. On occasion, contiguous areas may be infected by direct extension (e.g: sinusitis and otitis media from upper respiratory tract colonization) (Klein and Marcy 1995).

The major portals of entry for the organisms are the babies respiratory tract and the skin. In early onset disease often the proliferation of organisms in amniotic fluid prior to delivery, presents the major inoculum of organisms to the lungs of the infants. From the portals of entry the newborn baby's blood stream is invaded, either by

direct entry of pathogen through intact mucous membrane or cutaneous barriers; this occurs more readily when these barriers have been damaged by physical or anoxic injury. Blood stream invasion leads to two major consequences; the cascade of pathogenic events in septicemia and the hematogenous infection of distant sites. The pathogenic cascade of septicemia includes endothelial injury, release of inflammatory mediators, stimulation of the release of marrow phagocytic cells, circulatory failure secondary to septic shock, persistent fetal circulation and the development of disseminated intravascular coagulation with concomitant renal failure. If held in check by host defences, hematogenous infection of distant sites may occur. The most common of these include meningitis, osteomyelitis, endopthalmitis and endocarditis (Boyer 1992).

Host factors

It is not known why an infant with one or more predisposing factors is at high risk of sepsis. Microbial factors such as inoculum size and virulence of the organism undoubtedly are significant. Metabolic factors may also be important. Fetal hypoxia and acidosis may impede certain host defence mechanisms or allow localization of organisms in necrotic tissue (Klein and Marcy 1995). Hyperbilirubinemia (Rooney et al, 1971), hypothermia, acidosis and

inherited metabolic disorders like galactosemia, intramuscular iron administration and intravenous lipid administration to the infants, are associated with a significant increase in neonatal sepsis (Klein and Marcy 1995).

Susceptibility to infection is also increased in the neonate with:

1. A defect in host defences including immunologic deficits.
2. Defects in the integrity of the skin or mucosa, such as those occurring in an infant with meningomyelocele ; or
3. Absence of viscera related to defence against infection, such as congenital asplenia.

Umbilical cord as a focus of infection

The umbilical cord is a particularly common portal of entry for systemic infections in the newborn infant. The devitalized tissue of the cord is an excellent medium for bacterial growth, and the recently thrombosed umbilical vessels provide direct access to the blood stream (Klein and Marcy 1995).

Closure of umbilical vessels and subsequent aseptic necrosis of the cord begins soon after the infant takes the first breath. The umbilical stump acquires a rich flora of micro-organisms. Within hours it is colonized with large numbers of gram positive cocci, particularly staphylococcus species and shortly thereafter with fecal organisms as

well (Rotimi and Duerben 1981). These bacteria may invade the open umbilical wound, causing a localized infection with purulent discharge. In hospitalized infants, periumbilical cellulitis and fascitis have a high incidence of bacteremia, coagulopathy, shock and death (Klein and Marcy 1995).

IMMUNOLOGY

It has been recognized that the human fetus and neonate are at increased risk of infection due to pyogenic bacteria, viruses and certain intracellular organisms. Compared to term neonates, premature neonates are at even higher risk. The increased risk of infection is largely due to impairment or immaturity of both humoral and cellular functions in the fetus and neonate (English and Wilson 1992).

Humoral immunity

Placental transport of IgG provides the neonate with antibodies present in he maternal IgG. There is no transport of IgA and IgM across the placenta, so that the neonate is lacking in most of the bacterial antibodies to gram negative enteric organisms which are largely IgM molecules (Gotoff and Behrman 1970). But the rarity of septicemia in infants with IgM and IgA deficiency and the frequency of

gram positive septicemia suggest a role for other host defences. Thus, the increased risk of neonatal septicemia with gram negative organisms is probably not due to IgM deficiency (Gotoff and Behrman 1970).

In general neonatal B cells can differentiate into IgM – secreting plasma cells as efficiently as adult cells. Unlike adult B cells, neonatal B cells do not differentiate into plasma cells that secrete IgG or IgA. These functions are not mature until approximately two years (IgG) and five years (IgA) of age respectively. The inability of neonatal B cells to switch isotopes from IgM to IgG or IgA may reflect the limitations in the intrinsic capacity of the cell to produce certain isotopes as well as the impaired T- cell help (English and Wilson 1992).

In addition to quantitative differences there are qualitative differences in immunoglobulin synthesis by the newborn. Some of the circulating IgM in the neonate may be non-functional monomeric instead of the pentameric IgM (Allansmith et al 1968).

The neonate is able to mount an antibody response to most protein antigens (T cell dependent) after either immunization or infection. In contrast, the response to many polysaccharide antigens (T cell independent) is absent or severely blunted. This poor response to polysaccharide antigens correlates with immunity of the human

infant's splenic marginal zone – the area where the initiation of immune response to T-cell independent antigens occur (Timens et al 1989).

Cellular immunity

T-cell function : Because T cells influence the function of both immune and non-immune cells involved in the host response to infection deficits in T-cell function could result in increased susceptibility to all types of infectious agents. However, the most compelling evidence linking diminished T – cell function to the increased rate and severity of infection of the neonate is in the area of intracellular pathogens, both viral and non-viral and is related to the reduced production of a γ–interferon by neonatal T-cells.

Neutrophils : The diminished neutrophil storage pool, reduced production of neutrophils in response to infection and impaired delivery of these cells to the sites of infection, have all been implicated in the pathogenesis of overwhelming infection, due to pyogenic bacteria in the human neonate. In addition, functional abnormalities of the neonatal neutrophils probably contribute to the risk of these infections. Many of the reported deficits of neonatal neutrophils function appeared to result primarily from diminished levels of

opsonins (antibodies, complement fragments and perhaps fibronectin).

Mononuclear phagocytes : The delayed influx of monocytes to the site of inflammation may contribute to the increased risk of infection due to pyogenic bacteria. However, diminished monocyte / macrophage function is probably most important in the increased risk of infection of the neonate, due to intracellular organisms and viruses (English and Wilson 1992).

Conditions affecting neonatal immunity

a. Small for gestational age : The effects of prematurity on immune function are multiple. Intrauterine growth retardation (IUGR) appears to have a greater effect on T-cell function, than on other aspects of the immune system. The results of studies of immunoglobulin level of IUGR infants are variable, some showing normal or increased levels and others showing diminished levels (Lewis and Wilson 1995).

b. Hyperbilirubinemia : Decreased neutrophil microbicidal activity (Wright et al, 1975), diminished in-vitro lymphocyte proliferation and decreased antibody response subsequently to immunization but not naturally encountered antigens (Nejedla 1970), have been observed in infant with hyperbilirubinemia. Whether these were a

direct effect of bilirubin or related to factors leading to hyperbilirubinemia has not been determined. Bilirubin inhibits complement dependent serum bacterial activity in neonates with hyperbilirubinemia (Lewis and Wilson 1995).

PATHOLOGY

The autopsy finding in neonatal septicemia depend upon whether the infection becomes localized when no focus of inflammation is found in any organ, petchiae or ecchymoses may be seen on serosal surfaces (Gotoff and Beharman 1970).

The hallmark of neonatal sepsis is its disseminated nature. Large numbers of organisms can be found in virtually every tissue. The inflammatory response is often minimal even at the time of death in early onset disease, which frequently occurs in 2 to 3 days. In contrast, late onset disease, which is frequently manifested as meningitis, is accompanied by a significant inflammatory response. Infants in this age group with enterobacterial meningitis have large amounts of hemorrhagic necrosis of the central nervous system (Krugman and Katz 1981).

Finding typical of bacteremia such as multiple disseminated abscesses of similar size, purulent vasculitis and intravascular identification of bacteria, are evident only in a minority of infants.

Shock accompanying sepsis will some times cause superimposed findings, including intraventricular hemorrhage, hepatic necrosis, renal medullary hemorrhage, renal cortical or acute cortical necrosis and adrenal hemorrhage and necrosis. Evidence of disseminated intravascular coagulopathy may also be present (Klein and Marcy 1995).

CLINICAL MANIFESTATIONS

Signs of fetal distress may be the earliest indication of infection among infants with sepsis beginning at or soon after delivery. Poor feeding, diminished responsiveness or just 'not looking well', may provide the only evidence that infection is present. More prominent findings are respiratory distress, apnea, lethargy, fever or hypothermia, jaundice, vomiting, diarrhoea and skin manifestations, including petechiae, abscesses and sclerema.

Focal infection involving virtually any organ can occur in infants with sepsis. Signs of illness associated with septicemia will however, often mask evidence of localized infection. Thus, evaluation of infants with suspected bacteremia must include a careful search for primary or secondary focus such as meningitis, pneumonia, urinary tract infection, otitis media, septic arthritis, osteomyelitis or peritonitis (Klein and Marcy 1995).

Serious bacterial infections are very uncommon in infants without any clinical evidence of illness. Occasionally, bacteremia is without signs (Albers et al, 1966)

The temperature of the infant with sepsis may be elevated, depressed or normal (Messaritakis et al, 1990). Signs of respiratory distress, including tachypnea, grunting etc, are common and important findings in the infants suspected of having sepsis. Apnea is one of the most specific signs of sepsis, but usually occurs late. Clinical signs of cardiovascular dysfunction, that occurs in the absence of congenital heart disease are sensitive signs of sepsis (Klein and Marcy 1995).

Jaundice is present in approximately one-third of the infants with sepsis (Rooney et al, 1971). Hepatomegaly and rarely splenomegaly may be present. Gastrointestinal disturbances are common and significant early signs of sepsis (Klein and Marcy 1995).

Sequelae

Disabilities that could be regarded as related to sepsis were identified in 22% of children, who survived neonatal septicemia (Allven et al, 1978). Osteomyelitis and bone destruction, were noted in 8% of infants followed for upto 3 years (Bennet et al, 1989). Another study described recurrence of bacteremia in a few adequately treated early onset group B streptococcal disease (Klein and Marcy, 1995).

LABORATORY DIAGNOSIS

The clinical signs of neonatal sepsis are subtle and non-specific and often the diagnosis is difficult to establish on the basis of clinical findings alone. Hence, the laboratory has a very significant role to play in the diagnosis of neonatal septicemia.

The laboratory diagnosis of neonatal septicemia is made by actual demonstration of the causative agent / antigens. A variety of laboratory determinations can also be helpful in detecting infection in the newborns.

Demonstration of the causative agent

Although the 1980s and early 1990s were characterized by development of a plethora of antigen detection and nucleic acid probe assays, blood culture has remained the standard among laboratory tests, for the diagnosis of bacteremia (Campos et al, 1994).

Culture of blood

Isolation of pathogenic microorganisms from blood is the most specific method of diagnosis of neonatal septicemia. A variety of manual and instrument assisted methods have been devised to facilitate detection of microorganisms in blood by culture (Campos et al, 1994).

CULTURE OF OTHER BODY FLUIDS

CSF examination and culture: Because meningitis may accompany sepsis, examination of the CSF should be considered in any neonate who is to be treated for sepsis. Approximately one-quarter of infants with sepsis have accompanying meningitis (Klein and Marcy 1995).

In newborn infants with bacterial meningitis, cells may number in thousands and polymorphonuclear leukocytes predominate early in the course of disease. A gram stained smear of CSF should be examined for detection of bacteria and appropriate media should be inoculated with CSF. Sarff and colleagues detected organisms in gram stained smears of CSF in 83% of patients with group B streptococcal meningitis and 785 of patients with gram negative disease (Sarff et al, 1976).

Culture of urine : Urine should be considered for culture from infants before initiation of antimicrobial therapy for sepsis and is optimally obtained by bladder tap or catheter. Studies suggest that culture of urine yield limited information about the source of infection in infants with signs of sepsis in the first few days of life (Klein and Marcy 1995).

Culture of other foci of infection : The infant with septicemia may have umbilical sepsis, conjunctivitis, otitis media, pustules over the

skin and other foci of infection. When these foci are identified, appropriate culture should be done.

Diagnostic needle aspiration and tissue biopsy : Needle aspiration of an infectious focus in the lungs, pleural space, middle ear, pericardium, bones, joints and other sites provide immediate and specific information to guide therapy. Tissue biopsy should be considered if infection is suspected and more obvious foci cannot be sampled or an infectious focus has not been revealed. Biopsy of bone marrow or liver may assist in diagnosing occult infection (Klein and Marcy 1995).

RAPID TECHNIQUES FOR DETECTION OF MICROORGANISMS

Various rapid diagnostic techniques have been developed either to detect the microorganisms, their antigens, metabolic products or specific genetic sequences in the blood.

Buffy coat examination : The rapid diagnosis of bacteremia can be accomplished by identification of microorganisms in the stained buffy leukocyte layer of centrifuged blood of the neonates (Humphery 1944). Acridine orange, a fluorochrome that attacks to nucleic acids, has been used instead of the standard dyes (Parikh and Singh 1995).

Latex agglutination : Latex particle agglutination procedures are available to detect circulating antigens of many organisms including group B streptococcus, E.coli, H. influenzae type B, streptococcus pneumoniae and staphylococcal teichoic acids (Baron et al, 1994). Cross reactions with antigens of other organisms can occur, to give false positive reactions.

Limulus assay for endotoxin : This test is based on a gelation reaction between lysates of limulus (horse-shoe crab) amebocytes and bacterial endotoxin (Levin et al, 1970). The results of clinical studies are equivocal ; some studies have found significant correlation between a positive limulus test and positive results of blood cultures for gram negative bacteria (McCraken and Sarff 1976). Others have indicated that it is neither specific nor sensitive.

Other methods : Counterimmunoelectrophoresis as a method of identifying bacterial antigens, has been used successfully for detecting the antigens of various pathogens like S.pneumoniae and H. influenzae in body fluids (Klein and Marcy 1995).

Gas liquid chromatography, which detects the metabolic end products of microbial activity in serum, can be used to identify organisms especially the anaerobes.

Lysis filtration of blood, and subsequent staining of the filter surface, may prove to be a very sensitive and rapid method for the detection of circulating microorganisms (Baron et al, 1994).

Laboratory aids for diagnosis

For years, investigators have sought a test or a panel of tests able to identify septic neonates accurately and more rapidly than by isolation of microorganisms from specimens of body fluids or tissue that are normally sterile. Although results of some studies have been encouraging, isolation of the microorganisms remain the most valid method of diagnosing bacterial sepsis.

White blood cell counts and ratio

Total leukocyte counts may either be elevated or reduced in neonates with sepsis. Change in total neutrophil count and total non-segmented neutrophil counts are inconsistent and have not been useful for the diagnosis. Neutrophil ratios such as immature to total neutrophils and bound to segmented neutrophils have been evaluated by various authors (Powell and Marcy 1995) and found to be unpredictable of neonatal septicemia.

Acute phase reactants : In the presence of inflammation caused by infection, trauma or other cellular destruction, liver hepatocytes under the influence of interleukin – 1 (IL-1) rapidly synthesize large amount of certain proteins collectively known as acute phase reactants. Serum levels of these proteins usually raise together; and in general, the degree of change in one is proportional to the degree of change in the others. The exact role of acute phase reactants in the inflammatory process is unknown; most appears to be a part of a primitive non-specific defence mechanisms. Several acute phase reactants have been extensively evaluated in neonatal sepsis, including C-reactive protein (CRP); fibrinogen and other proteins that influence the erythrocyte sedimentation rate; haptoglobin and α_1–acid glycoprotein (Powell and Marcy, 1994).

C-reactive protein

Discovery of CRP was reported in 1930 by Tillet and Francis.

Pepys (1982) described C-reactive protein as acute phase reactant. Acute phase reactants are group of plasma proteins, concentration of which increases following most forms of tissue injury or inflammation or association of malignant neoplasia.

While a number of acute phase reactants have been described for early diagnosis of neonatal septicemia such as fibrinogen, C-

reactive protein, orosomucoid and pre-albumin, majority of them have low sensitivity. CRP, however, is a notable exception and therefore, been found clinically applicable as reported by Sann et al. (1984).

Osmond et al (1977), described structure of CRP and proposed that molecule of human CRP (mole weight 105,500) is composed of five identical non-glycosylated polypeptide units which are non-covalently associated in a disc like configuration with cyclic pentameric symmetry. Hurliman et al, (1966) reported that CRP is synthesized by hepatocyte and no evidence is obtained of its production by any other cell type.

Claus et al (1970), reported that there was a rapid rise in C-reactive protein concentration from normal level by as much as 3000 fold in response to acute tissue injury, inflammation or infection.

Klindmark (1971), reported that CRP enhance phagocytosis of various bacterial species by peripheral blood leucocytes in a serum free medium.

CRP is a potent activator of complement system when reacting with capsular polysaccharide and choline phosphatadine. According to Seigal et al. (1975) CRP has the power to combine with T-lymphocyte and inhibit certain of their functions. It can also bind with Fe receptor

of macrophages to bring about phagocytosis by acting as an opsonin as has been suggested by Mortenson et al (1977).

The normal levels of CRP are not precisely defined. Following variety of acute inflammatory stimuli or tissue necrosis a sharp rise in serum concentration of CRP is noted within few hours. No other acute phase protein with the exception of SAP (Serum amyloid P component) is known to increase in concentration so markedly.

Peltola (1984), reported that value upto 20 µg/ml may be considered as normal. Philips et al (1886), suggested a value of more than 8 µg/ml as positive. Chandra et al (1988), also considered level of CRP above 8 µg/ml to be positive for infection. Netteke et al (1992), in their study considered CRP levels more than 10 µg/ml to be positive for infection while Sharma et al (1993) considered the value of more than 6 µg/ml as abnormal.

Increase CRP production is a very early and sensitive response to most forms of microbial function. Several pathological events such as bacterial infection, a few viral infection, myocardial infarction, connective tissue disease and surgical trauma results in increased CRP levels. Some elevation is found occasionally in a few physiological conditions such as pregnancy and puerperium.

Systemic infection in newborns are often difficult to detect because of subtle clinical signs. An elevated CRP is an early and

sensitive indicator of neonatal septicemia. When the infection is controlled with adequate chemotherapy, CRP levels rapidly fall. In fact CRP values are at present the best indicator for the assessment of effectiveness of antimicrobial. A supranormal plateau without a complete return to normal should be regarded as warning sign relapse or other complications.

Desai et al (1982), observed high sensitivity (87.5%) and specificity (83.3%) for CRP test among four parameters viz. Band/Total neutrophil, leucocytes less than 5000/cc, Micro-ESR and CRP, they used.

Hindocha et al (1984), reported that eleven out of twelve infants of proven sepsis had raised serum CRP which decreased significantly after successful treatment.

Sanne et al (1984), reported elevated CRP levels in 85% cases of neonatal sepsis.

Kalra et al (1985), estimated CRP in various neonatal infections. They found that CRP is equally effective diagnostic tool as blood culture in neonatal septicemia, while in superficial infection, it is not of much importance.

Singh et al (1987), in their study found CRP is most sensitive (80%). and specific (91%) screening test for neonatal sepsis.

Chandna et al (1988), in their study of neonatal septicemia showed that the CRP test had 83% sensitivity, 42% specificity and 57% positive predictive accuracy. They concluded the CRP test to be the most useful rapid diagnostic test when taken singly.

Apart from a proven value in the diagnosis, attempts have been made to utilize CRP estimation for follow up of neonatal septicemia by Sanne et al (1986). He reported early decline of CRP and rapid return to normal to be indicative of good response to treatment.

Ali et al (1988), reported a study on the prognostic value of CRP in neonatal septicemia. Initial mean ± SD CRP levels in culture positive and culture negative cases were 18.97 ± 8.54 µg/ml and 18.93 ± 10.59 µg/ml respectively. Serial estimation of CRP were done. CRP levels showed a significant decline as early as the third day of therapy among those who survived, while the decline in expired cases was not significant. Among the survivors the CRP levels at the end of therapy was comparable to that of controls.

Suri et al (1991), reported a comparative study of the prognostic utility of CRP, α-1 antitrypsin and α-2 macroglobulin in neonatal septicemia. Serial estimation of CRP was done. The initial mean value of CRP in the study group was 12 µg/ml, 12 to 24 hours after the first sample the mean value was 17.4 µg/ml and after a week of therapy the mean value was 17 µg/ml among the survivors. The levels of CRP

at the corresponding periods were much lower in those who died. This study was in strict contrast to the study conducted by Ali et al (1986). Suri et al (1991), described the high levels of CRP on the seventh day of illness among the survivors to exert a protective influence. Suri et al (1991) concluded, CRP used as a screening test in detection of septicemia, had a very low yielding index; on the contrary CRP estimation had considerable utility for predicting outcome in terms of survival. They had thus suggested that predicting outcome was the most important clinical use of CRP in neonatal septicemia.

Netteke et al (1992), reported a prospective evaluation of the impact of perinatal conditions like perinatal asphyxia, premature rupture of membrane, respiratory distress syndrome and periventricular and intraventricular hemorrhage on CRP production. These perinatal conditions were hypothesized to cause an acute phase response and provoke a non specific increase in CRP. But Netteke's study found no relationship between CRP production and the perinatal condition and concluded that the CRP measurement to be a discriminative test for bacterial infection in the neonate.

Sharma et al (1993), reported diagnostic and prognostic utility of CRP in neonatal septicemia. They estimated CRP in septicemia neonates on 1, 7, 14 days and found that on first day CRP was raised in all cases of septicemia, however, on fourteenth day it touched zero

among those babies who had improved however, in babies who deteriorated or died the values showed an increasing tendency.

Berger C et al (1995), reported that during 1st 3 days of life CRP, leucopenia, neutropenia, were comparably good test, while after 3 days of life CRP was the best single test in early detection of neonatal septicemia. Serial CRP estimation confirm the diagnosis, monitor the course of infection and efficacy of antibiotic treatment.

Jurges et al (1996), - Reported CRP is useful test in early diagnosis of neonatal septicemia and may be useful in differentiating between bacterial and viral etiology.

Benitz et al (1998), - Reported that serial CRP levels are useful in diagnostic evaluation of neonates with suspected infection. Two CRP level < 1 mg/dl obtained 24 hrs apart, 8 to 48 hours after presentation, indicate that bacterial infection is unlikely. The sensitivity of a normal CRP at initial evaluation is not sufficient to justify withholding antibiotic therapy. The positive predictive value of elevated CRP level is low, especially for culture proven early onset infection.

De A et al (1998), reported that 111 cases had positive CRP out of 200 cases of septicemia.

Kuster et al (1998), - Reported that CRP could allow earlier initiation of antibiotic therapy with corresponding improvement in outcome in very low birth weight infants with sepsis.

Fibrinogen, haptoglobin and orosomucoid

These acute phase reactants tend to increase in neonatal septicemia as well as in other non-infectious conditions. Normal values are sometimes observed even with serious infection. Hence, they were found to be unreliable indicators of neonatal septicemia.

Erythrocyte sedimentation rate

Although ESR eventually becomes elevated in most infants with sepsis, delay in the raise can occur in many conditions. Therefore, it is of little significance in the initial diagnosis of neonatal septicemia (Powell and Marcy 1995).

Immunoglobulin – M (IgM)

IgM is the largest of the polymeric immunoglobulins, usually being a pentamer of the H_2 L_2 structure with one J chain. IgM immunoglobulin has a sedimentation constant of 19S, this fraction, like IgA globulin is incapable of crossing the placenta, but its synthesis can occur at a slow rate in the fetus. After birth, the rate of synthesis

increases rapidly and it has been reported that adult level may be reached by the 9th month of age. Recent works have shown that the newborn infants are by no means immunologically incompetent as it was once thought to be.

Molecular weight of IgM is 900,000 Daltons. IgM is also known as macroglobulin due to its high molecular weight. IgM is produced in the primary response to antigenic challenge. IgM is the main immunoglobulin produced by the fetus and while the amount formed is usually small when there is fetal infection, substantial IgM response may occur. Serum concentration of IgM in a newborn is about 1.6 to 31 mg% (Hardy et al, 1969), which rapidly increases to adult level of 50 – 150 mg% by about one year of age. The IgM level in serum is between 5 and 10 percent of the total antibody protein. Following are the cord serum levels of IgM as reported by different workers – 1.6 to 31 mg% (Hardy et al, 1969), 0 – 25 mg% (Khan et al, 1974), 9.12 ± 10.04 mg% (Kaur et al, 1979), 22.8 – 84.4 mg% (Hariharan et al, 1984) and 12.1 ± 13.5 mg% (Sharma et al, 1986).

The immunoglobulin-M can be produced by the fetus by 10 ½ weeks of gestation (Gotoff, 1974) but the level remains very low at birth (Steihm et al, 1966). AllanSmith et al (1968), reported that level of IgM remains constant for the first 5 days after birth, and then increases rapidly for 2 days. Newborn develop 50% level of the adult

values of IgM by the end of four months of age and adult levels are attained by the age of 1 to 2 years (Allan Smith et al, 1968). Some of the newborns were having high levels than normal infants, which was attributed to maternal bleeding into fetal circulation (Sever et al, 1969). Levels of IgM are not related to sex. Evons et al (1971), reported similar levels of IgM among multiple-birth newborns corresponding to those of single infant or same gestational age. Raghvan et al (1976) reported a higher level of IgM in the Indian population as compared to the reports from the west. IgM levels were low and had no relation with the gestational age and birth weight indicating absence of placental transfer and negligible synthesis by the fetus (Mehambare et al, 1978). Higher serum IgM levels in cord blood as compared to Western World, were reported by Hariharan et al (1984). Sharma et al (1986), reported no significant relationship of cord blood IgM with birth weight and gestational age. A high value of serum IgM was observed in the post-mature babies by Goel et al (1987). Low serum IgM values at birth were reported by Kolhatkar et al (1987), and they increase as the age advances.

Alford et al (1967), evaluated from their study of acutely infected neonates postnatally, that high levels of IgM beginning from the third day after onset of the infection.

Sheldon et al (1969), in another study of 57 cases suffering from septicemia observed that serum IgM levels were raised in all the cases, the rise was first to appear in newborns with pneumonia.

John L Sever (1969), reported that IgM immunoglobulin levels are often elevated in infants in association with congenital and perinatal infections.

Hardy et al (1969), reported an increase in the levels of serum IgM in newborns having septicemia as well as in those whose mother's had suffered from respiratory infections during pregnancy.

Prasad et al (1971), observed an appreciable rise in the serum IgM fractions in response to infection. They studied twenty normal and fifteen neonates with acute infections, and observed serum IgM values in controls and infection group to be 17.88 ± 1.77 mg% and 39.2 ± 8.36 mg% respectively.

Blankenship et al (1974), also observed an increase of IgM in 80% of cases and reported a greater increase in viral as compared to the bacterial infections. Further, they reported that staphylococci were most antigenic among various bacteria's they studied.

Malik et al (1977), studied the immunological profile of neonates exposed to the risk of perinatal infections. They found that the levels of serum IgM were significantly raised in newborns born to mother's having definite history of acute infection, during pregnancy. The levels

of IgM were normal in neonates born after premature rupture of membranes. The failure of IgM levels to increase in the neonates born after premature rupture of membranes was explained by the authors to be due to the failure of the infective stimulus to reach the fetal immune system. The values of IgM in control and infected group newborns were 11.38 ± 6.76 and 98.7 ± 58.7 mg% respectively.

Khatua et al (1984), studied humoral immunity, morbidity and mortality from infective diseases of 50 newborns and reported that cord serum IgM values were significantly raised (> 20 mg%) in infants whose mother's had infective ailments during pregnancy.

Mehta et al (1987), studied serum IgM levels in 70 septicemic and 40 normal neonates to evaluate its usefulness as a diagnostic and prognostic tool in neonatal septicemia. The workers, reported significantly higher serum IgM levels in cases of septicemia than in their control group of cases.

Material & Methods

Material and methods

The present study was carried out in the Department of Pediatrics in collaboration with Department of Microbiology. M.L.B. Medical College, Jhansi.

Selection of cases:

Newborns below the age of 1 month were included within study. Newborns were divided into two group :

(1) Study group

(2) Control group

Neonates presenting with symptoms and signs suggestive of septicemia were included in the study group.

Symptomatic neonates were taken from department of pediatrics presenting with at least 3 or more of following criteria were included in the study :-

- Prematurity / low birth weight
- Birth asphyxia needing resuscitation
- Prolong labour more than 24 hours
- Prolong rupture of membrane more than 24 hours
- Refusal to feed/ poor feeding

- Lethargy, hypothermia / hyperthermia, abdominal distention, irregular respiration, vomiting, apneic spells, convulsion / full fontanelle, diarrhoea, hepatosplenomegaly, pallor, cyanosis, jaundice, bleeding tendency, sclerema, umbilical discharge.

Selection of control

Asymptomatic newborns were taken from department of Obstetrics and Gynecology served as control group.

A detailed obstetrics history was inquired from the mother, which included history of fever, toxemia of pregnancy, anti partum hemorrhage, rash, diabetes, hypertension, multiple pregnancy, any systemic disease, duration of leaking P/V.

Detailed history and examination of each newborn was carried out, which included status of cry, any resuscitation intervention, gestational age, birth weight, prelacteal feed if given, feeding habits, age at onset of symptoms.

Above history was recorded on a pre-designed proforma.

Before administering any antibiotics, 2 to 3 ml of blood was obtained from a peripheral vein of the neonate aseptically.

Following investigations was done-

- Hb
- TLC

- DLC
- Blood culture
- Estimation of C-RP
- IgM estimation

Other tests were done on specific indication

- Chest X-ray
- Serum bilirubin
- CSF examination
- Gastric aspirate cytology
- Urine examination, routine and culture as and when required.
- Culture from any septic foci and other investigations as and when required.

All the above investigations were done in department of pediatrics, department of pathology and department of microbiology.

Estimation of IgM was done by radial immunodiffusion technique using commercially available kit from the binding site.

Specimen collection and preparation

Immediately after admission, blood samples were taken, about 2.5 – 3 ml of blood was taken by venepuncture from every case by aseptic method from a peripheral vein.

Blood culture was sent immediately after admission in every suspected case of neonatal septicemia. Blood for culture was obtained from peripheral veins after cleaning the site with spirit and povidone iodine. About 1 ml of blood was withdrawn by means of a sterlized needle and syringe. Blood in syringe was transferred to the culture bottle.

The remaining 1.5 – 2 ml of blood was placed in a sterile glass vial and was allowed to clot. The serum was separated from the clot by centrifugation using sterile precautions. The clear serum separated was then transferred to a sterile screw capped vial. A total of 57 serum samples thus collected, were used for the serological tests namely, CRP test and IgM estimation.

Serological tests

The C-reactive protein test and immunoglobulin –M (IgM) estimation was done on the 57 serum samples collected.

C-reactive protein test

CRP was estimated by Latex agglutination slide test

PRINCIPLE OF TEST

CRP slide test for detection of CRP is based on principal of agglutination. Uniform latex particles are coated with anti human

CRP. The serum containing CRP on mixing with latex reagent agglutinates, showing a positive test result. A quantitative CRP test was performed by the slide agglutination method using commercially available kit.

The CRP was done soon after the serum was separated by the rapid Latex agglutination method. The test kit was obtained from Tulip diagnostics. C-reactive concentrations of greater than 0.6 mg / dl in the test serum gives a visible agglutination with this kit. A positive and negative control was included with each batch of sample tested. All of the samples that showed visible agglutination within two minutes were considered positive and the others as negative.

Immunoglobulin–M estimation

The IgM estimation was done by the standard Mancini technique of the single radial immunodiffusion method, using commercially available kit from "The Binding Site Ltd.", Birmingham, UK.

PRINCIPLE OF TEST

The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate monospecific antibody. Antigen antibody complexes are found which under the right conditions, will form a precipitate ring. The ring size will increase until

equilibrium is reacted between formation and breakdown of these complexes, this point being termed 'completion'. At this stage, a linear relationship exists between the square of the ring diameters and the antigen concentration. By measuring the ring diameters produced by a no. of samples of known concentration, a calibration curve may be constructed. The concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample on reading off the calibration curve.

Based upon the ideal linear calibration curve, a reference table is provided with the kit, which converts ring diameters directly to protein concentrations.

Test procedure

- By using micropipette 5 µl volume of each test serum was plated within separate wells of Radial immunodiffusion plates.
- Similarly 15 control sera obtained from healthy newborns at birth were run on antibody agar slide. These were used as normal controls.
- After the serum samples were added to the wells, the lid of RID plates were tightly closed and the plate stored flat with the lid uppermost at room temperature (approximately 20 – 24° C). The minimum incubation time was 72 hours for IgM.

On completion of immunodiffusion time, the diameter of the rings were measured with slide callipers using a magnifying lens.

- The concentration of immunoglobulin in each test sample were read directly from the RID reference table.

Observations

Observations

The present study was conducted in the Department of Pediatrics, in collaboration with Department of Microbiology, M.L.B. Medical College, Jhansi from October 2003 to October 2004.

The study comprised of 57 neonates, clinically suspected of septicemia, admitted in the Department of Pediatrics of M.L.B. Medical College, Jhansi and 15 healthy neonates were taken as controls.

The various observations made during the study are tabulated as follows:

The distribution of neonatal septicemia according to the age at onset of symptoms, is shown in Table 1.

Table 1

Incidence of septicemia related to the time of onset of the symptoms.

Type	Age at onset	Number	Percentage (%)
Early onset	0 – 7 days	45	79.13 %
Late onset	8 – 28 days	12	20.87 %

Out of 57 suspected cases of septicemia 45 (79.13%) had symptoms in the first week of the life, i.e. early onset septicemia, rest of 12 cases (20.87%) came after that, i.e. late onset septicemia.

Table 2a

Distribution of study and control group according to sex.

Sex	Study group		Control group	
	No.	%	No.	%
Male	37	64.91 %	10	66.66 %
Female	20	35.09 %	5	33.33 %

Male infants accounted for 37 cases (64.91%), while female infants were 20 in number (35.09%) in study group, while in control group male infants accounted for 10 cases (66.66%), while female infants were 5 in number (33.33%).

Table 2b

Place of delivery of newborns in study group.

Place of delivery	No.	Percentage (%)
M.L.B. Medical College, Jhansi	34	59.64 %
Other hospitals	13	22.82 %
Home delivery	10	17.54 %
Total	57	100 %

Majority of cases, 34 out of 57 (59.64%) were delivered at M.L.B. Medical College, Jhansi, while 22.82% were delivered at other hospitals and 17.54% were of home delivery.

Table 3a

Distribution of study and control group according to gestational age.

Gestational age (weeks)	Study group		Control group	
	No.	%	No.	%
Less than 37	34	59.6 %	5	33.33 %
More than 37	23	40.4 %	10	66.66 %

Table 3b

Gestation wise distribution of early and late onset septicemia

Gestational age (weeks)	Early onset septicemia		Late onset septicemia	
	No.	%	No.	%
Less than 37	19	73.07 %	7	26.93 %
More than 37	8	66.66 %	4	33.33 %
Total	27	71.05 %	11	28.95 %

In study group, out of 57 newborns, 34 (59.6%) were born before 37 weeks of gestation, while 23 (40.4%) were born at term. In control group 5 newborns (33.33%) were born before 37 weeks of gestation, while 10 (66.66%) were born at term.

Table 4

Distribution of study and control group according to body weight.

Weight (Kg)	Study group		Control group	
	No.	%	No.	%
Less than 1	1	1.75 %		
> 1 to < 2	22	38.59 %	3	20 %
2 to < 2.5	24	42.10 %	8	53.33 %
≥ 2.5	10	17.54 %	4	26.66 %

Analysis of birth weight showed that in study group 46 babies (82.46%) were of low birth weight. While 10 babies (17.54%) were more than 2.5 Kg. In control group out of 15 babies, weight of 11 babies (73.34%) were below 2.5 Kg and 4 babies were more than 2.5 Kg.

Table 5a

Clinical conditions associated with neonatal septicemia.

Condition	Number	Percentage %
Jaundice	15	26.31 %
Hypothermia	12	21.05 %
Meconium aspiration syndrome	4	7.01 %
Hemolytic disease of newborn	3	5.26 %
Respiratory distress syndrome	3	5.26 %
Neonatal seizures	3	5.26 %

Jaundice was the commonest clinical condition associated with neonatal septicemia, observed in 15 neonates followed by hypothermia in 12 cases. Meconium aspiration syndrome was seen in 4 neonates, Hemolytic disease of newborn, respiratory distress syndrome and neonatal seizures were the other conditions associated with neonatal septicemia.

Table 5b

Frequency of symptoms in study group

Symptoms	No.	Percentage (%)
Refusal of feeds / poor feeding	47	90 %
Lethargy	43	81 %
Feeble cry	40	76 %
Diarrhoea	33	60 %
Respiratory distress	17	30 %
Abdominal distension	17	30 %
Vomiting	13	24 %
Apnoeic spells	9	16 %
Convulsion	7	12 %

The commonest symptom was refusal of feeds or poor feeding (90%), while the symptoms with least frequency was convulsion (12%).

Table 6

Complication of delivery in the mothers of neonates with septicemia.

Complication	Number	Percentage %
Prolonged labour	3	5.26 %
Meconium stained liquor	12	21.05 %
Prolonged rupture of membrane for >24 hrs	18	31.57 %
Premature rupture of membranes	20	35.08 %
Intra partum fever	4	7.01 %

As revealed in Table 6, premature rupture of membrane was the most common feature in 20 cases (35.08%), followed by prolonged rupture of membranes for > 24 hours in 18 cases (31.57%), meconium stained liquor in 12 cases (21.05%) than intra partum fever in 4 cases (7.01%) and prolonged labour in 3 cases (5.26%).

Table 7

Foci of infection in neonatal septicemia.

Infection	Number	Percentage %
Single Foci		
Umbilical sepsis	28	49.12 %
Conjunctivitis	26	45.61 %
Abscess / Wound/ Cellulitis	2	3.50 %
Pyoderma	1	1.75 %
Multiple Foci		
Umbilical sepsis and Conjunctivitis	9	
Umbilical sepsis, Conjunctivitis & pyoderma	1	
Pyoderma and Conjunctivitis	1	

Umbilical sepsis was the commonest focus of infection occurring in 49.12 % neonates followed by conjunctivitis (45.61%). Abscesses were present in 2 cases. Multiple foci of infection was seen in 11 cases. Nine neonates had both conjunctivitis and umbilical sepsis, whereas had Umbilical sepsis, Conjunctivitis and pyoderma.

Table 8

Systemic involvement in neonates with septicemia.

Illness	Number	Percentage %
Meningitis	20	35.08 %
Sclerema	5	8.77 %
Pneumonia	4	7.01 %
Urinary tract infection	1	1.75 %

Systemic involvement of the meninges, skin and lungs were common. Meningitis was present in 20 (35.08%) cases, sclerema in 5 (8.77%) cases and pneumonia in 4 (7.01%) cases. Urinary tract infection was observed in one case.

Table 9

Results of blood culture in study and control group.

Blood Culture	Study group		Control group	
	No.	%	No.	%
Positive	38	66.66 %		
Negative	19	33.34 %	15	100 %
Total	57	100.00 %	15	100 %

In study group out of 57 blood samples subjected to culture, 38 samples yielded growth, giving a culture positivity rate of 66.66%. The remaining 19 samples (33.34%) yielded no growth of organisms

The various bacteria isolated from the cases of neonatal septicemia is shown in table 10.

In control group, out of 15 blood samples, result of blood culture in all cases were negative.

Table 10

Bacterial isolates from neonatal septicemia

Organism	Number	Percentage	Type			
			Early onset septicemia		Late onset septicemia	
			No.	%	No.	%
Gram Negative bacilli (n = 33) 86.84%						
Klebsiella	16	42.10 %	12	48.0 %	4	50 %
Pseudomonas	12	31.57 %	10	40.00 %	2	25 %
E.Coli	5	13.15 %	3	12.0 %	2	25 %
Gram Positive cocci (n = 5) 13.15%						
Staphylococcus	5	13.15 %	2	40.0 %	3	60.0 %

Gram negative bacilli were isolated in 33 cases (86.84%), while 5 isolates (13.15%) were of gram positive cocci.

Klebsiella was the predominant organism isolated. Out of 16 cases, 12 were of early onset septicemia and 4 were of late onset septicemia.

Pseudomonas was the next common organism present in 12 isolates, out of 12 (31.57%) 10 were of early onset type and 2 were of late onset type.

E.coli was isolated in 5 cases, out of which 3 cases were of early onset type and two cases were of late onset type.

Staphylococcus was the only gram positive cocci causing septicemia in the present study, accounting for 5 cases (13.15%), out of which 2 cases were of early onset type and 3 cases were of late onset type.

Table 11a

Incidence of mortality related to blood culture

Culture results	Total Number	Mortality	
		Number	Percentage %
Culture positive	38	27	71.05 %
Culture Negative	19	4	21.05 %
Total	57	31	54.38 %

Table 11b

Culture results	Total Number	Mortality	
		Number	Percentage %
Gram Negative bacilli	33	25	65.78 %
Gram Positive cocci	5	2	34.22 %
Total	38	27	100 %

Among 38 neonates who were culture positive, 27 expired (71.05%). Gram negative bacilli infection resulted in higher mortality (65.78%), then gram positive coccal infection (34.22%). The mortality among the culture negative cases was 21.05%.

Table 11c

Relation of place of delivery with blood culture positivity and mortality

Place of delivery	No.	Positive blood culture		Mortality	
		No.	%	No.	%
M.L.B. Medical College, Jhansi	34	26	76.47 %	18	69.23 %
Other hospitals	13	4	30.77 %	2	50 %
Home delivery	10	8	80.0 %	7	87.5 %

26 (76.47%) out of 34 neonates delivered at M.L.B. Medical College, Jhansi, showed positive blood culture, while 4 (30.77%) out of 13 neonates delivered at other hospitals had positive blood culture. 8 (80%) out of 10 home delivered babies showed positive blood culture. Mortality was more among hospital born neonates.

Table 12

Results of CRP test and blood culture in study and control group

Blood culture	Total	CRP test			
		Study group		Control group	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Positive	38	26 (68.42%)	12 (31.57%)		
Negative	19	9 (47.36%)	10 (52.6%)	-	15 (100 %)
Total	57	35 (61.40%)	22 (38.59%)		

The CRP test was positive in 26 out of 38 (68.42%) bacteriologically proved cases of septicemia. In the 19 bacteriologically negative cases CRP was positive in 9 (47.36%) cases. In control group all 15 cases were bacteriologically negative and also in all of them CRP test was negative.

Table 13

Results of IgM estimation and blood culture in study and control groups.

Blood culture	Total	IgM levels			
		Study group		Control group	
		>20 mg/dl (%)	<20 mg/dl (%)	>20 mg/dl (%)	<20 mg/dl (%)
Positive	38	22 (57.89%)	16 (42.10%)		
Negative	19	3 (15.78%)	16 (84.21%)		15 (100 %)
Total	57	25 (43.85%)	32 (56.14%)		

An IgM level of more than 20 mg/dl was found in 22 out of 38 bacteriologically proved cases of septicemia (57.89%). IgM was also raised above 20 mg/dl in 3 out of 19 cases bacteriologically negative cases (15.78%).

In control group, IgM level in all 15 cases were below 20 mg/dl.

The outcome of neonatal septicemia was analyzed in relation to the IgM levels in bacteriologically proved cases of septicemia (Table 14).

Table 14a

Outcome of neonatal septicemia in relation to IgM levels.

IgM levels	Total	Outcome	
		Expired (%)	Improved (%)
> 20 mg/dl	22	3 (13.63%)	19 (86.36%)
< 20 mg/dl	16	8 (50%)	8 (50%)
Total	38	11	27

Three newborns out of 22 who had IgM ≥ 20 mg / dl (13.63%) expired, while 8 newborns out of 16 with IgM < 20 mg/dl expired (50.0 %).

Table 14b

Outcome of neonatal septicemia in relation to CRP levels.

CRP levels	Total	Outcome	
		Expired (%)	Improved (%)
> 0.6 mg/dl	26	10 (38.46%)	16 (61.54%)
< 0.6 mg/dl	12	2 (16.66%)	10 (83.34%)
Total	38	12	26

Ten newborn out of 26 who had CRP test positive (38.46%) expired while 2 newborns out of 12 CRP test negative expired (16.66%).

Table 15

Relation of combination of CRP test and IgM estimation to blood culture.

Blood culture	CRP and IgM (≥ 20 mg/dl)					
	Study group			Control group		
	Total	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)
Positive	38	27 (71.05%)	11 (28.94%)	0	0	0
Negative	19	3 (15.78%)	12 (84.21%)	15	0	15 (100%)
Total	57			15		

Both CRP and IgM (≥ 20 mg/dl) were positive in 27 out of 38 bacteriologically proved cases of septicemia (71.05%). They were also positive in 3 out of 19 bacteriologically negative cases (15.78%).

All the control neonates had CRP level below 6 µg/dl and IgM level below 20 mg/dl.

Table 16

Sensitivity, specificity and positive predictive accuracy of CRP and IgM

Parameter	Sensitivity	Specificity	Positive predictive accuracy
CRP	68.97 %	53.57 %	60.61 %
IgM	61.54 %	82.14 %	77.27 %
CRP + IgM	70.0 %	86.67 %	87.5 %

Sensitivity of CRP was 68.97%, while specificity was 53.57% and positive predictive accuracy was 60.61%. For IgM sensitivity was 61.54%, while specificity was 82.14% and positive predictive accuracy was 77.27%. When bot CRP and IgM test combined sensitivity was 70.0%, while specificity was 86.67% and positive predictive accuracy was 87.5%.

Discussion

Discussion

The present study was conducted in the Department of Pediatrics, M.L.B. Medical College, Jhansi. One of the commonest clinical entity confronting a neonatologist in our country is neonatal septicemia. The grave prognosis and the urgent need to start the appropriate treatment in neonatal septicemia calls for its accurate and rapid diagnosis.

The study was aimed to assess clinical profile of neonatal septicemia, to study the diagnostic utility of Immunoglobulin-M estimation and C-reactive protein detection in neonatal septicemia and to evaluate the predictive value of IgM and C-reactive protein in outcome of neonatal septicemia.

In the present study, neonatal septicemia occurred in the first week of life in 79.13% of the neonates. Septicemia occurred after 8 days in 20.87% of neonates (Table 1). These observations reflect the immaturity of the immunologic responses in the neonate in the first few days of life (English and Wilson, 1992). A high incidence of septicemia has also been observed by other authors in the first week of life. Bhakoo et al (1980), found 80% of cases occurring within first seven days of life. Khatua et al (1986), reported 70.64% septicemia developing within 5 days of life. Vesiker et al (1985)

reported 43.9% cases occurring within the first 24 hours of life and 90.24% cases occurring in the first week of life.

Neonatal septicemia was more common in the male infants (64.91%) in the present study (Table 2a), other studies have also observed a similar sex predilection. Khatua et al (1986), found 70.7% incidence in males, while Chandna et al (1988) reported 58%. Genetic factors have been postulated for this increased preponderance in males (Washburn et al, 1965). Another factor of male preponderance is that the male infants are better cared than female in our society. So, they are brought to the hospital more in number than female infants.

The 59.64% cases of suspected neonatal septicemia were born at M.L.B. Medical College, Jhansi, whereas 40.36% babies were born at other hospitals or at home. Home born infants formed 17.54% of cases (Table 2b). Home born infants have a high risk of developing septicemia and other infections due to delivery under unhygienic conditions. The sterility of the instruments used to cut the umbilical cord is always in doubt, more so often the delivery is conducted by untrained personnel (Khatua et al, 1986). Bhakoo et al (1974), noted that 61% of the neonates studied by them were hospital born and 39% home born; similar to the present study.

Majority of the infants (85.83%) were born by vaginal delivery, while LSCS and instrumental deliveries accounted for 8.66% and 5.51% respectively. Babies are prone for injury, which can get infected and lead to septicemia. Macfarlane (1987), noted delivery by caesarian section in 8% and instruments in 4%of neonates with septicemia, which is similar to our present observations.

Anemia was the commonest complication of pregnancy in the mothers of the neonates studied. Anemia in the pregnant mother leads to low birth weight or small for date babies, who are at increased risk of developing sepsis.

The mother's of 42 – 52% of the newborns studied, gave a history of one or more complications of delivery. Prolonged labour, meconium stained liquor, prolonged rupture of membranes for more than 24 hours, premature rupture of membranes were the commonest (Table 6). Under these circumstances ascent of the maternal genital flora can occur and cause fetal and neonatal infection (Benirschke et al, 1960). Meconium stained liquor may increase the chances of fetal infection (Klein and Marcy, 1995). Raghavan et al (1992), observed prolonged rupture of membranes (> 24 hours) in 18% and meconium stained liquor in 46% of mothers of neonates with sepsis.

Mishra et al (1988), Boyle et al (1983) and Anand et al (1991) reported leaking P/V of more than 24 hours in 68.5%, 86% and 55.9% of cases of neonatal septicemia respectively. Prolonged rupture of membrane of more than 24 hours have also been shown by Takkar et al (1974); Bennet et al (1981); and Khatua et al (1986), to be contributory to a high incidence of bacteriologically positive cases of septicemia.

Regarding the birth weight of study group, it was found that incidence of low birth weight in our study was 82.46% (Table 4). Our findings are in agreement with that of Phillip et al (1980), who reported that incidence of low birth weight babies was 68.2% in neonatal sepsis. Increased incidence of neonatal septicemia in low birth weight babies have also been reported by Namdeo et al (1985), Khatua et al (1986), Chugh et al (1988), and Mathur et al (1991).

Low birth weight babies have low IgG due to poor placental transport. Thymus is atrophic in neonates with IUGR and T cells are remarkably diminished resulting in poor cellular immunity. Levels of C_3, C_4, factor B, lysozyme and interferon are lower than normal. There is also poor neutrophilic function in terms of chemotaxis, phagocytosis and intracellular killing. These factors are responsible

for a higher incidence and mortality in low birth weight neonates (Yoder et al, 1986 and Chandana et al, 1988).

In our study refusal of feeds was the commonest symptom (90%). In the decreasing order of frequency the symptoms were lethargy (84%), feeble cry (76%), diarrhoea (60%), respiratory distress (30%), abdominal distension (30%), vomiting (24%) and apnoeic spells (16%). Convulsion was the presenting symptom in only one case (Table 5a).

Khatua et al (1986), also observed refusal of feeds as the commonest symptoms (92.3%) and cases with convulsion to be the least in frequency (10.8%). Chandana et al (1988), observed lethargy as the commonest symptom (70%) in contrast to respiratory distress with a frequency of only 12%. Mondal et al (1991), divided his population of study into two groups depending upon place of delivery. He reported lethargy (94%) in hospital delivered and refusal of feeds (84%) in home delivered neonates as the commonest symptoms.

Abdominal distension and convulsions were associated with highest incidence of death in our study. Gotoff and Behrman (1970); Guha et al (1978); Namdeo et al (1985) and Khatua et al (1986) also reported similar findings.

Nearly two-third of the neonates in the present study were born before 37 weeks of gestation (59.6%) (Table 3a). A birth weight of 2000 gms or less was found in 38.59% of newborns (Table 4). Premature babies are usually born with lower weight and the two conditions are interrelated (Singh 1991). Both these conditions can predispose neonate to infection. These infants are at a clear disadvantage owing to an immature defence system, and have a diminished T-cell function compared to mature infants (Bhaskaran et al, 1977). Many authors have recorded an increased incidence of septicemia in preterm and low birth weight babies. Bhakoo et al (1974), and Khatua et al (1986) found that 51% and 63% of their cases were premature respectively. The incidence of septicemia in newborns of birth weight < 2000 gms was 55% and 55.43% respectively. These figures are in close agreement with that of the present study.

Thus, premature constituted a sizeable proportion of cases who had septicemia. Chandana et al (1988), studied cases of neonatal septicemia in order to establish an early diagnosis in such cases. Author reported that 36% cases of neonatal septicemia in their study were premature. However, Saxena et al (1978), observed prematurity in only 21% of such cases. A significantly higher incidence of septicemia in premature infants is probably due

to the lack of inherent defensive mechanism in them, both at cellular and humoral level. It may be due to relatively longer stay and excessive exposure of premature babies to diagnostic and supportive procedures in the hospital. Low birth weight infants have low IgG and are more susceptible to infection, while placental transport of IgG from maternal to fetal circulation increases with the maturity, this transport is hampered in small for date infants, who are often the product of placental insufficiency (Chandana et al, 1988). Among premature cases, 73.07% cases (19) presented as early onset septicemia, while rest 26.93% cases (7) presented as late onset septicemia. While in fullterm neonates, 66.66% cases (8), had symptoms in 1st week of life i.e. early onset septicemia and rest 28.95% cases (11) were presented as late onset septicemia.

Birth asphyxia was observed in 21.65% of the babies. Birth asphyxia causes immunological insult (Raghavan et al, 1992). The use of resuscitative equipment tend to expose the neonate to pathogenic microbes (Klein and Marcy, 1995). Birth asphyxia was noted in 28% of neonates with septicemia by Raghavan et al (1992), which is almost similar to the present study.

Jaundice was the commonest clinical condition associated with septicemia, followed by hypothermia than meconium aspiration syndrome (Table 5a). Reduced immunological activity has been

observed in neonates with hyperbilirubinemia (Wright et al, 1975; Lewis and Wilson, 1995). Jaundice may also be a result of infection (Klein and Marcy, 1995). Jaundice was observed by Mishra et al (1995) in 29% and Khatua et al (1986) in 38% cases respectively. Meconium aspiration indicates fetal distress, a risk factor for sepsis (Singh, 1991); and meconium in the liquor may promote bacterial growth (Klein and Marcy, 1995). Thus a high incidence of sepsis is seen in this condition.

The above findings were in accordance with studies of Gotoff and Behrman (1970); Bhakoo et al (1980); Namdeo et al (1985) and Khatua et al (1986).

The commonest focus of infection in the present study was umbilical sepsis, seen in 49.12% of cases (Table 7). Umbilical sepsis is a well known focus of infection and it is more common where unsterile methods are used to cut the cord at birth. Saxena et al (1980), also found that umbilical sepsis was the commonest focus of infection (21.42%). Their study group was home born neonates, in whom unsterile cord division is common, and explains the higher incidence of sepsis. Chandana et al (1988), found umbilical sepsis in 16% of neonates studied by them.

Meningitis was the commonest complication occurring in 35.08% neonates (Table 8). It is due to hematogenous spread of

infection to meninges (Boyer, 1992). Bhakoo et al (1980), observed meningitis in 8% neonates with septicemia, while Choudhary et al (1987), recorded an incidence of 5.33% neonates.

The overall mortality rate was 54.38% in the present study (Table 11a). A high mortality rate in neonatal septicemia has been observed universally. Khatua et al (1986), found 57.6% mortality, while Chugh et al (1988), observed 53.3% mortality in neonatal septicemia. These observations are quite similar to the present findings. While Vesikari et al (1985), observed 23% mortality in Finland, Ohlsson et al (1986) in Saudi Arabia and Tessin et al (1990), in Sweden recorded 33% and 15% mortality rate from neonatal septicemia. The neonatal care facilities in the above countries is better than that available in most hospitals in India. Thus, the mortality rate from neonatal septicemia in those countries is much less, compared to that in India. In present study, mortality among Gram negative bacilli constituted 65.78%, while 34.22% mortality demonstrated in Gram positive cocci (Table 11b).

In the present study, 26 out of 34 neonates (76.47%) delivered at M.L.B. Medical College, Jhansi, showed positive blood culture and 66.66% mortality (Table 11c). Mathur et al (1996), reported that septicemia in home delivered babies was 47.9%, while in hospital born babies the septicemia was found in 52% of the

cases. Positive blood culture was more in hospital delivered neonates, than in home delivered neonates. In our study, positive blood culture were in 8 neonates, out of 10 home delivered neonates, and mortality was 87.57% (7 cases) in home delivered cases. Namdeo et al (1987), also observed a higher incidence of blood culture positivity in hospital delivered neonates (59.5%). Home born infants have a high risk of developing septicemia and other infections due to delivery under unhygienic conditions.

Blood Culture

A blood culture positivity rate of 66.66% was observed in the present study. Klebsiella was the predominant isolates constituting 42.10% followed by P. aeruginosa (31.51%), E.coli (13.15%) and staphylococcus (13.15%).

The commonest causative organisms in the Indian studies include Klebsiella (Khatua et al, 1986; Chugh et al, 1988), E.coli (Bhakoo et al, 1974) and S.aureus (Mishra et al, 1985).

Thus, the literature on the etiology of neonatal septicemia has no uniformity ; the predominant organisms being different in various places. It is evident that Gram negative bacilli are the commonest organisms in India. Even in the study of Mishra et al, Gram negative bacilli as a whole, outnumber the Gram positive cocci, though S.aureus is the most common organism.

The mortality to the culture positive cases were 71.05% compared to only 21.05% in culture negative cases (Table 11a). Increased mortality in blood culture positive cases have also been observed by Guha et al (1978), Namdeo et al (1986), Khatua et al (1986), and others. Higher mortality in culture positive cases were due to invasion of blood stream by large number of bacteria.

In our study Gram negative organism were responsible for the bulk of septicemia in both early onset and late onset cases. Chowdhury et al (1975), Guha et al (1978), Monga et al (1986) observed a predominance of Gram negative organisms.

As in the other studies, Gram negative bacilli from the majority of the isolates in the present study. Klebsiella is the commonest organism in the present study, similar to the results of Khatua et al (1986) and Chugh et al (1988). One of the reasons expounded for the predominance of an organism in causing septicemia in the nursery is the selective pressure of antimicrobial agents. The resistant microorganisms tends to colonize and proliferate in the nurseries (Klein and Marcy, 1995). This is also true with Klebsiella infections (Montgomerie and Ota, 1980). In our hospital, Klebsiella that are highly resistant to most of the antimicrobial agents are commonly encountered in many other specimens like pus, urine etc. Thus, cross contamination and nosocomial transmission may play a

significant role in the etiology of Klebsiella septicemia (Gupta et al, 1993). The frequency of P. aeruginosa and S.aureus septicemia in the present study also point towards nosocomial transmission of infections.

P. aeruginosa is the second most common organism isolated in the present study, followed by E.coli and S.aureus. Studies of Bhakoo et al (1986), have also found a similar pattern.

Ohlsson et al (1986), from Saudi Arabia, also reports the predominance of Gram negative bacilli; Klebsiella followed by E.coli. Saudi Arabia, although economically sound, is still a developing country and may be experiencing the same conditions as in India. Hence, a similar pattern of organisms is seen.

Gluck et al (1966), from U.S.A also reported a predominance of Gram negative bacilli, the Gram positive cocci forming less than one-tenth of the isolates. Thus, the situations of 1966 in the U.S are being experienced in India now, as far as the etiology of neonatal septicemia is concerned. Later, reports from the West shows that group B Streptococci have emerged as the single most common pathogen in neonatal septicemia as reported by Vesikari et al (1985) and Tessin et al (1990). The antimicrobial therapy has again been postulated for the predominance of this organism in the developed countries (Macfarlane, 1987). The increased colonization

of the genital tracts of women with group B Streptococci causes increased incidence of early onset septicemia (Vesikari et al, 1985).

In the present study Klebsiella was the predominant isolates constituting 42.10% (16) cases, out of which 12 cases presented as early onset and rest 4 were presented as late onset septicemia (Table 10).

Pseudomonas was the second most common organism isolated in the present study constituting 31.57% cases (12), out of which 10 cases presented as early onset and rest 2 cases as late onset septicemia.

3 cases out of 5 (13.15%) presented as early onset septicemia in E.coli positive cases while rest 2 as late onset septicemia.

Among 5 positive cases of staphylococcus (13.15%), 2 presented as early onset and 3 presented as late onset septicemia.

The overall mortality in the present study was 54.38%, Gram negative infection accounting for 65.78% and Gram positive infection 34.22%. Among the Gram negative infections, Klebsiella infection had the highest mortality (59.34%). Though Klebsiella was the important cause of mortality in both early and late onset septicemia, it was lower in early onset septicemia. Similarly, the

mortality was higher (67.3%) in low birth weight infants than the normal birth weight infants (45.95%).

C-Reactive Protein Test

CRP, an acute phase reactant is found to be elevated in neonatal infection and value return to normal with clinical improvement and hence been designated as simple, effective and early test for the diagnosis of neonatal sepsis.

In the present study, CRP test was positive in 68.42% in blood culture positive cases of the patients studied. Present result is in correlation with many other workers. Sabel et al (1974), found increased CRP in 85.7% cases of neonatal septicemia. Hindescha et al (1984), found significantly raised concentration of CRP in eleven out of twelve cases. Kalra et al (1985), observed positive CRP test in 95% cases of neonatal septicemia. Sann et al (1986), found CRP positivity in 86% while Bhatia et al (1988), found CRP positivity in 87.5% cases of septicemia. Sharma et al (1993), observed positive CRP test in 82% cases of neonatal septicemia.

Sann et al (1986), in study of neonatal septicemia observed elevated CRP level in 86% cases of neonatal septicemia. He reported early decline of CRP and rapid return to normal level to be indicative of good response to treatment, whereas, persistent

elevation of serum CRP suggested that either treatment was inadequate or some complication had developed.

Ali et al (1988), in his study on the prognostic significance of CRP in neonatal septicemia showed a significant decline in CRP, early in the course of the disease in survivors after institution of therapy. While the decline in cases who later expired was insignificant.

Sharma et al (1993), showed a significant decline in CRP early in the course of disease in survivors.

The CRP test was positive in 61.40% of the patients studied. Among them 68.42% were culture positive (Table 12). The sensitivity of CRP test was 68.97%, while the specificity was 53.57%. A 60.61% positive predictive accuracy was found for CRP test in the present study.

Philip and Hewitt (1980), observed sensitivity of CRP test was 47%, while the specificity was 86% and 22% positive predictive accuracy.

Chandna et al (1988), noticed 83% of sensitivity and 42% of specificity and 57% of positive predictive accuracy.

Sann et al (1984), noted a poor CRP response in infants less than 24 hours of life. Increases in CRP levels are also associated with non-infectious conditions such as birth asphyxia, respiratory

distress syndrome, intra cerebral hemorrhage and meconium aspiration syndrome (Powell and Marcy, 1995).

Parikh and Singh (1995), observed sensitivity of CRP test was 83%, while the specificity was 42% and 57% positive predictive accuracy.

IgM estimation

The finding of raised levels of immunoglobulin–M in response to infections have been uniformly observed by several other workers – Alford et al (1967), 13 – 130 mg%; Hardy et al (1969), 30 mg%; Khan et al (1969), 55 mg%; Prasad et al (1971), 39.2 ± 8.36 mg % and Malik et al (1977), 98.7 ± 58.7 mg%. The elevated levels of immunoglobulin IgM as a response to infection is possibly an exaggeration of the normal response to a myriad of antigens in the extra uterine environment.

Alford et al (1967), evaluated from their study of acutely infected neonates postnatally, that high levels of IgM beginning from the third day after onset of infection.

Sheldon et al (1969), in another study of 57 cases suffering from septicemia observed that serum IgM levels were raised in all the cases, the rise was first to appear in newborns with pneumonia.

John L Sever (1969), reported that IgM immunoglobulin levels are often elevated in infants in association with congenital and perinatal infections.

Hardy et al (1969), reported an increase in the levels of serum IgM in newborns having septicemia as well as in those whose mothers had suffered from respiratory infections during pregnancy.

Prasad et al (1971), observed an appreciable rise in the serum IgM fractions in response to infection. They studied twenty normal and fifteen neonates with acute infections and observed serum IgM values in control and infection group to be 17.88 ± 1.77 mg% and 39.2 ± 8.36 mg% respectively.

Blankenship et al (1974), also observed an increase of IgM in 80% of cases and reported a greater increase in viral as compared to the bacterial infections. Further, they reported that staphylococcus was the most antigenic among various bacteria's, they studied.

Malik et al (1977), studied the immunological profile of neonates exposed to the risk of perinatal infections. They found that the levels of serum IgM were significantly raised in newborns born to mothers having definite history of acute infections during pregnancy. The levels of IgM were normal in neonates born after premature rupture of membranes. The failure of IgM levels to

increase in the neonates born after premature rupture of membranes was explained by the authors to be due to the failure of the infective stimulus to reach the fetal immune system. The values of IgM in control and infected group were 11.38 ± 6.76 and 98.7 ± 58.7 mg% respectively.

Khatua et al (1984), studied humoral immunity, morbidity and mortality from infective diseases of 50 newborns and reported that IgM values were significantly raised (> 20 mg%) in infants whose mother had infective ailments during pregnancy.

Mehta et al (1987), studied serum IgM levels in 70 septicemic and 40 normal neonates to evaluate its usefulness as a diagnostic and prognostic tool in neonatal septicemia. The workers, reported significantly higher serum IgM levels in cases of septicemia than in their control group of cases.

IgM levels of ≥ 20 mg/dl were observed in 43.85% of patients studied. Among them 57.89% were culture positive. The sensitivity of the test was 61.54% and specificity 82.14%. The positive predictive accuracy was 77.27%.

Alford et al (1967), noticed 84.78% sensitivity and 94.32% specificity. The positive predictive accuracy was 79.59%.

Philip and Hewitt (1980), obtained 61.22% sensitivity and 87.66% specificity of the test. The positive predictive accuracy was 70.25%.

Although, a significant proportion of infants with sepsis show elevated levels of IgM, it may remain normal in upto half of all infants with sepsis. There may be 2 to 7 days delay in the raise of IgM depending on the age at which the first symptoms appears (Blankenship et al, 1969). In addition, viral infections, minor localized bacterial infections and meconium aspiration syndrome were associated with a significant increase in IgM concentration (Pourcyrous et al, 1991). Early treatment of bacterial infection can alter the immunological load, thereby affecting the IgM levels (Blankenship et al, 1969).

Among the newborns, having proven septicemia and elevated IgM levels, only 13.63% expired, while 50% of neonates with low levels of IgM expired (Table 14a). These results emphasize the role of IgM as bactericidal antibodies, in the prognosis of neonatal septicemia. The estimation of IgM can thus predict the prognosis of the neonate with septicemia. Hardy et al (1969), observed a death rate 20 times higher in neonates with low IgM levels than in those with elevated levels.

Ten newborn out of 26 who had CRP test positive i.e. > 0.6 mg/dl expired (38.46%), while 61.54% improved with CRP positive test. Two newborns out of twelve with CRP negative test i.e. < 0.6 mg/dl expired, which constituted 16.66%, rest 83.34% improved with CRP negative test (Table 14b).

As neither of the two tests : CRP test and IgM estimation were found to be reliable when used alone (Table 14), and as the sensitivity of the individual tests were not high, statistical analysis was done to find out the usefulness of the two tests in the diagnosis of neonatal sepsis when considered together (Table 15).

The tests were together positive in 71.05% of blood culture positive cases. The sensitivity increased to 70% and specificity to 86.67%. A great increase in the positive predictive accuracy to 87.5% occurred, with a significant fall in the false negatives (Table 16).

Thus, when the two tests were considered together, more accurate diagnosis could be made. These tests are most useful when confirmatory diagnostic inference of septicemia is needed, in view of ambiguous clinical signs and blood culture results.

Summary

Summary

The present study was conducted in the Department of Pediatrics and its neonatal unit in collaboration with the Department of Microbiology, at M.L.B. Medical College, Jhansi, over a period of 12 months from October 2003 to October 2004.

The aims of this study were : To study the clinical profile of neonatal septicemia, to study the diagnostic utility of immunoglobulin–M estimation and C-reactive protein detection in neonatal septicemia, and to evaluate the predictive value of IgM and C-reactive protein in outcome of neonatal septicemia.

Selection of study group was done on the basis of clinical symptomatology suggestive of sepsis, later confirmed by blood culture.

Premature infants and low birth weight babies were in majority (82.46%). Many newborns had a history of birth asphyxia.

Male babies (66.66%) with septicemia outnumbered the females (33.33%). Associated physical conditions like jaundice, meconium aspiration syndrome and others were present in a significant number of newborns.

The commonest presenting symptom was refusal to feed or poor feeding. The symptoms associated with highest incidence of death were convulsion and abdominal distension.

Umbilical sepsis was the major focus of infection (49.12%) followed by conjunctivitis. Meningitis was the most frequent localized lesion followed by sclerema and pneumonia.

Neonatal septicemia occurred in the first week of life in 79.13% of the neonates studied, septicemia occurred after 8 days in 20.87% of the neonates.

The 59.64% cases were born at M.L.B. Medical College, Jhansi, whereas 40.36% babies were born at other hospitals or at home. Home born infants formed 17.54% of the cases.

Anemia was the commonest complication of pregnancy in the mother's of the neonates studied. Anemia in the pregnant mother leads to low birth weight or small for date babies, who are at increased risk of developing sepsis.

The mother's of 42 – 52% of the newborns studied gave a history of one or more complication of delivery. Prolonged labour, meconium stained liquor, prolonged rupture of membranes for more than 24 hours, premature rupture of membranes were the commonest.

More than half of the neonates in the present study, were born before 37 weeks of gestation (59.60%).

Birth asphyxia was observed in 21.65% of the babies.

Jaundice (26.31%) was the commonest clinical condition associated with septicemia, followed by hypothermia in 21.05% neonates than meconium aspiration syndrome in 7.01% neonates.

The overall mortality rate was 54.38% in the present study.

A blood culture positivity rate of 66.66% was observed in the present study. Klebsiella was the predominant Gram negative bacilli constituting 42.10% of the isolates, followed by pseudomonas aeruginosa 31.57% and E.coli 13.15%. Among Gram positive cocci - staphylococci was the sole organism (13.15%) isolated. The CRP test was positive in 61.40% of the patients studied. Among them 68.42% were culture positive. The sensitivity of CRP test was 68.97% while the specificity was 53.57%. A 60.61% positive predictive accuracy was found for CRP test in the present study.

IgM levels of ≥ 20 mg/dl were observed in 43.85% of patients studied. Among them 57.89% were culture positive. The sensitivity of the test was 61.54% and specificity 82.14%. The positive predictive accuracy was 77.27%.

Among the newborns having proven septicemia and elevated IgM levels, only 13.63% expired, while 50% of neonates with low

levels of IgM expired. The estimation of IgM predict the prognosis of the neonates with septicemia. Death rate higher in neonates with low IgM levels, than in those with elevated levels.

Neither of the two tests : CRP test and IgM estimation were found to be reliable when used alone. When the tests were considered together more accurate diagnosis could be made.

The tests were together positive in 70% of blood culture positive cases. The sensitivity increased to 70% and specificity to 86.67%.

These tests are most useful when confirmatory diagnostic inference of septicemia is needed, in view of ambiguous clinical signs and blood culture results.

Conclusion

Conclusion

- Significant risk factors associated with the development of sepsis in a neonate were premature rupture of membrane > 24 hours, prematurity, low birth weight and resuscitation required by newborns.
- Clinical feature of sepsis remained non-specific vague and subtle. Refusal to feed, lethargy, fever, hypothermia, apnea were the clinical features most commonly associated with sepsis neonatorum. Temperature changes were significant to note that they were present in all the cases.
- The commonest presenting symptoms were refusal to feed or poor feeding. The symptoms associated with highest incidence of death were convulsion and abdominal distension.
- The commonest sign was Jaundice and then hypothermia. The sign associated with the higher incidence of death was sclerema.
- Premature infants and low birth weight babies were in majority. Many newborns had a history of birth asphyxia.
- Male babies with septicemia outnumbered the females.
- Associated physical conditions like jaundice, meconium aspiration syndrome and other were present in a significant number of infants.

- Umbilical sepsis was the major focus of infection followed by conjunctivitis.
- Meningitis was the most frequent localized lesion followed by sclerema and pneumonia.
- A positive blood culture was obtained in 66.66% cases. Gram negative bacilli were the major pathogens. K. pneumoniae was the commonest causative organism accounting for 42.10% of the isolates. Pseudomonas aeruginosa (31.57%) was the next important pathogen followed by E.coli and staphylococcus.
- An overall mortality rate of 54.38% was recorded in the present study. In neonates with positive blood cultures the mortality was 71.05%.
- A rapid and accurate diagnosis of neonatal septicemia cannot thus be overemphasized. Two rapid, non-culture tests analyzed in the present study were the CRP test and IgM estimation. The CRP test had a 68.97% sensitivity, 53.57% specificity and a positive predictive accuracy of 60.61%.
- The IgM estimation showed a sensitivity of 61.54%, specificity of 82.14% and positive predictive accuracy of 77.27%.
- The CRP test with low specificity and IgM estimation with low sensitivity, were found to be unreliable as a diagnostic tool by themselves. However, the IgM estimation was a good prognostic

indicator in culture proven sepsis. As IgM estimation is a specific test, it can give confirmatory evidence of infection.

- The combination of CRP test and IgM estimation had a sensitivity of 70%, specificity of 86.67% and positive predictive accuracy of 87.5%, all of which are more than those of the individual test. Thus, a combination of the two tests can help in an early and accurate diagnosis of neonatal septicemia.

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Working Proforma

NEONATAL HISTORY:

GESTATIONAL AGE :

BIRTH WEIGHT :

PRELACTEAL FEED IF GIVEN : YES/NO.

FEEDING HABIT : BREAST FED / TOP FED / BOTH

AGE AT ONSET OF SYMPTOMS :

OTHER

PRESENTING COMPLICATIONS :

EXAMINATION OF NEW BORN :

GENERAL EXAMINATION :

WEIGHT OF BABY : SKIN :'NORMAL/ABNORMAL

G.C : ANY POSITIVE FINDING ON

H.R. : THE SKIN.....

R.R

UMBLICUS : HEALTHY / UNHEALTHY

TEMPERATURE	:	FONTANELLE	:
HYDRATION	:	SKULL	:
PALLOR	:	SPINE	:
ICTERUS	:	EYES	:
CYNOSIS	:	EARS	:
OEDEMA	:	OTHERS	:
CLUBBING	:		

SYSTEMIC EXAMINATION :

RESPIRATORY SYSTEM :

CARDIOVASCULAR SYSTEM :

ABDOMINAL EXAMINATION :

CENTRAL NERVOUS SYSTEM :

ANY CONGENITAL ANOMALY :

OTHERS :

WORKING PROFORMA

Topic : Immunoglobulin-M estimation and C-reactive protein detection in neonatal septicemia

NAME: BHT NO.:

AGE: WARD/BED NO.:

SEX: DATE/TIME OF ADMISSION:

D.O.B: DATE/TIME OF DISCHARGE/DEATH:

RELIGION:

ADDRESS

EDUCATIONAL STATUS:

OF MOTHER: ILLITERATE / JUST LITERATE / FORMAL EDUCATION

OF FATHER: ILLITERATE / JUST LITERATE / FORMAL EDUCATION

SOCIO-ECONOMIC STATUS OF FAMILY:

G.....P.....A..

PREVIOUS PREGNANCY AND OUTCOME OF SIBLINGS:

ANTINATAL HISTORY:

H/O FEVER. : YES/NO,

H/O TOXEMIA OF PREGNANCY : YES/NO.

H/O ANTIPARTAM HEMORRHAGE : YES/NO.

DURATION :

H/O RASH : YES/ NO.

H/O DIABETES : YES/NO

H/O HYPERTENSION : YES/NO.

H/O MULTIPLE PREGNANCY : YES/NO.

H/O ANY SYSTEMIC DISEASE : YES/NO.

DURATION OF LEAKING P/V :

COLOUR OF LIQUAR : COLORLESS / MUCONIUM STAINED
BLOOD STAINED /ANY OTHER.

OUDOUR OP LIQUAR : ODORLESS/FOUL SMELLING.

NUMBER OP P/V DONE :

HISTORY OP VIRGINAL INFECTION :

NATAL HISTORY :

ONSET OF LABOUR : MATURE/ PREMATURE CAUSE OF
PREMATURE.LABOUR

DURATION OF LABOUR : 1ST STAGE..... 2ND STAGE.....

NATURE OF DELIVERY : CS / NORMAL / INSTRUMENTATION

DETAIL OF VIGINAL DELIVERY : VERTEX / BREECH / FACE /
TRANSVERSE / OTHER.

INDICATION OF C.S. / ASSISTED :
DELIVERY

ANY COMPLICATION DURING LABOUR: YES / NO
(IF YES, DETAIL.....)

PLACE OF DELIVERY : HOME / HOSPITAL
AT HOME DELIVERY CONDUCTED BY.....

POSTNATAL HISTORY:

APAGAR SCORE : 1 MIN....5 MIN....10 MIN....20 MIN.

CRY : IMMEDIATE / DELAYED.... MINUTES
AFTER BIRTH

ANY RESUSCITATION : YES/NO.
/ INTERVENTION IF YES, DETAIL.....

TWIN DELIVERY : YES/NO.

NATURE PLACENTA :